



LIBRARY
New Delhi

Call No. _____

Acc. No. 7567

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or after _____

Return Date	Return Date

book, he shall be required to replace
the book or pay its price, if detected
at the time of return.

**HELP US TO KEEP THIS BOOK
FRESH AND CLEAN**

CEREAL CHEMISTRY

Published by the American Association of Cereal Chemists

<i>Editor-in-Chief</i>	-	-	-	M. J. BLISH
<i>Assistant Editor</i>	-	-	-	R. T. PRESCOTT
<i>Managing Editor</i>	-	-	-	R. M. SANDSTEDT

Associate Editors:

MARY M. BROOKE	CLINTON L. BROOKE
C. O. SWANSON	W. F. GEDDES



VOLUME XV—Nos. 1-6
JANUARY-NOVEMBER, 193



Lancaster, Pennsylvania

1938

LANCASTER PRESS, INC., LANCASTER, PA.

CEREAL CHEMISTRY

VOL. XV

JANUARY, 1938

No. 1

A STUDY OF THE TOXICITY AND SELENIUM CONTENT OF SELENIFEROUS DIETS: WITH STATISTICAL CONSIDERATION¹

KURT W. FRANKE and E. PAGE PAINTER²

Department of Experiment Station Chemistry, South Dakota State College,
Brookings, South Dakota

(Received for publication February 24, 1937)

Robinson's (1933) discovery of selenium in grains which Franke (1934) had found toxic greatly accelerated progress in the study of the disturbance colloquially known as the "alkali disease." Selenium is generally considered to be the toxicant in the foodstuffs but one can not overlook the possibility that molybdenum and tellurium (Beath, Eppson, and Gilbert, 1935), chromium, vanadium, and arsenic (Byers, 1934), and other poisonous metals may be taken up by plants in amounts which would render them dangerous when used as food for animals.

Franke and Potter (1935) fed selenium salts to albino rats and found the effects practically identical with those produced by seleniferous grains. Painter and Franke (1935) removed the selenium from toxic protein hydrolysates and Franke and Painter (1935) found the selenium-free product nontoxic when fed. Franke, Moxon, Poley and Tully (1936) produced monsters in chick embryos by injecting selenium salts into eggs. Some of these abnormalities were apparently identical with those Franke and Tully (1935, 1936) produced by feeding hens toxic grain. There was, however, a wider variation in types of monsters and greater involvement of organs when the natural toxicant was fed.

Due to these differences and apparent differences observed in gross pathology in rats fed experimental diets containing selenium, the question arises: Were the observed results due to the different selenium

¹Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication No. 26 from the Department of Experiment Station Chemistry; also is part 18 of "A New Toxicant Occurring Naturally in Certain Samples of Plant Foodstuffs." These investigations are being carried out under the Purnell Fund.

²Present address: Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

compounds or to additional effects of other poisons? Beath and coworkers (1934) and Draize and Beath (1935) observed different external pathology when different seleniferous plants were eaten. They attribute this to the different compounds of selenium and in some cases to the secondary action of other poisons.

Beath (personal communication) states that the toxicity of certain plants grown in Wyoming does not vary according to selenium content. This would be expected inasmuch as Beath, Draize, Eppson, Gilbert, and McCreary (1934) and Franke and Painter (1935, 1936) have shown that different forms of selenium occur in plants. Although it has not been conclusively proved that selenium is the sole toxicant, other toxicants, if present, may produce symptoms similar to those of selenium. Furthermore, they must have certain chemical properties in common with selenium.

When the first reports on the effects of toxic grains on animals appeared, the selenium content had not been determined quantitatively. The selenium content of the samples which have been collected and bio-assayed during the past six years of experimentation has now been determined. Selenium has been found in all samples which were found toxic.

If, by a comparison of the toxicity of the different samples, it is found that there is a difference in toxicity, other poisons than selenium or selenium compounds of different toxicity must be present. By a comparison of the toxicity of groups of diets it should be possible to ascertain whether or not specific kinds of cereals always metabolize selenium compounds of the same toxicity. If the toxicity of selenium salts added to an otherwise normal diet is included in this comparison, the relative toxicity of naturally occurring (organic) selenium and inorganic selenium may be determined.

There may be a seasonal variation in the toxicity of the samples. Steyn (1934) finds the presence of most plant toxicants profoundly affected by climatic conditions during the growing season. The metabolism of selenium compounds may also depend upon the growing conditions. If a seasonal variation does occur, it should be revealed by a comparison of toxicity of samples collected over a period of years.

Many residents of the selenium area believe that the cereal grains decrease in toxicity after maturity. Owing to the firmness with which selenium is bound in the protein of cereals (Franke and Painter 1935, 1936), this idea at first appears fatuous. Consideration should be given, however, to the observations of stockmen in the seleniferous area. Some stockmen cling so firmly to their belief that the grains decrease in toxicity that they store the grains for several seasons before feeding them.

Experimental

A summary of results of feeding toxic diets was taken from the records of five years of experimentation. The method of setting up feeding trials has been adequately described in former publications (Franke, 1934, 1934a; Franke and Potter, 1934). The different seleniferous diets fed have different compositions, as shown in Table I.

TABLE I
SELENIFEROUS DIETS FED

	Composition of diets		
	I	II	III
Whole ground grain	70%	82%	82%
Casein	11	10	10
Sucrose	15		
Lard	2		3
Cod-liver oil	x	2	2
Dried yeast	x	2	2
McCollum's salt mixture		4	1
Salt (NaCl + CaCO ₃)	2		

x = given yeast and cod-liver daily separate from diet.

Although selenium salts have not been added to corn or barley diets, they have been added to several synthetic diets and the conclusion (unpublished) has been reached that there is no difference in toxicity when the different food components vary in amounts no greater than in the diets reported in this paper.

One problem confronting nutrition workers is what responses by the animal best determine the adequacy or inadequacy of a diet. Is it possible to control all the variables so that over a number of years of feeding an absolute value can be assigned each of the several diets used? If this can be accomplished and applied to the diets containing selenium, a direct comparison of the toxicity with the selenium content is at hand.

Before a comparison of the toxicity of different diets can be made, criteria, expressed in mathematical terms, must be chosen upon which to determine relative toxicity. Franke (1934, 1934a), Franke and Potter (1934, 1935), and Franke and Painter (1935) have considered the incidence of death, growth rate, restriction of food consumption, general appearance, gross pathology, and, in some cases, hemoglobin levels, as evidences of the toxicity of a diet. No mathematical comparison is possible with the last three manifestations of selenium poisoning given.

From a toxicological viewpoint, the incidence of death would be the best measure of toxicity. In many cases where the selenium content

was low, no deaths occurred, so death alone can not be taken as the sole measure of toxicity.

The depression of growth by seleniferous feeds is a good criterion of toxicity and it is of practical importance to the stockman. Franke (1935) has studied the effect of small amounts of corn in diets. When toxic corn containing 28 p.p.m. of selenium was 17.5% of a diet, there was depression of growth. The growth rate alone may not show the comparative toxicity of different cereals, or even of like cereals when diets of slightly different composition are used. In nearly all feeding trials a parallel group of control animals was run. There has been some variation in the growth rate of the control groups. The uncertainty of using growth rate of the experimental group can then be partly obviated by taking the difference in growth between the control and experimental animals as a measure of toxicity.

Franke (1934), and Franke and Potter (1935, 1936) have considered the voluntary restriction of food consumption by rats on seleniferous diets a good measure of toxicity. A comparison of toxicity, based on food consumption, should also take into consideration the food consumed by the control group. The average daily food consumption of the control groups on the different diets was not the same. Therefore, the difference between the food consumption of the control and experimental was also used.

For fair interpretation of the results, cognizance must be taken of observations from feeding seleniferous diets. The age of the rats is one evident factor in the effect of seleniferous grains on animals. Franke and Potter (1936a) and Schneider (1936) have found that older animals are much more resistant. Older animals fail to grow satisfactorily, but a few days' difference in age at the time of first feeding selenium has a great effect on the life span.

Females are more susceptible to selenium poisoning than males. The incidence of death from feeding grains and selenium salts is shown in Table II. These data were taken from comparable series in which litter mates and an equal number of males and females were in each group. Although the difference in life span is not great, there is a definite trend.

Nearly all analyses were made on the different lots of grain after part of the lot had been fed. Byers (1935) has shown that samples taken from the same field may vary several-fold in selenium content. This factor was not recognized in the early work, so there is the possibility that the samples were not homogeneous. Such a possibility would probably not cause great error because the samples would ordinarily be fairly well mixed when harvested. In order to overcome this problem, all samples with numbers above 568 were thoroughly

mixed when received. The selenium was determined on air-dried samples by a slight modification of the method described by Robinson, Dudley, Williams, and Byers (1934).

TABLE II
RELATIVE SUSCEPTIBILITY OF MALES AND FEMALES TO SELENIUM POISONING

Series	Source of selenium	Diet number	Rats	Number of deaths during period							
				0-30		30-50		50-70		70-100	
				m.	f.	m.	f.	m.	f.	m.	f.
8	No. 388 corn	I	40	3	3	2	3	4	5	6	6
66	No. 582 wheat	II	10	1	4	4	1				
66	No. 583 wheat	II	10	1	1	4	2		2		
94	No. 663 corn	III	10	1	0	1	4	1	0	1	
94	No. 664 corn	III	10					1	2	1	1
62	No. 570 corn	II	10	1	2	2	3	3	1		
33	No. 459 wheat	II	10	0	3	3	1	1	1		
107	Na ₂ SeO ₄	III	10			1	2	2	2	3	2
107	Na ₂ SeO ₃	III	10	1	4	0	0	2	1	1	1
Total dead by end of period (Grains)				9	15	28	32	37	42	38	44
Total dead by end of period (Se salts)				1	4	1	5	5	8	9	11
Total dead by end of period (All rats)				10	19	29	37	42	50	47	55

The laboratory conditions of the feeding trials varied somewhat during the five-year period. Temperature and humidity are now controlled better than they were in the first experiments.

A summary of the results from the feeding of seleniferous diets is shown in Table III. Only those manifestations of selenium poisoning which could be measured in absolute terms are included in the table. The more factors included in the comparison of toxicity the more accurate will be the results, because there will be less chance for individual differences of animals to alter results and to restrict interpretations.

The measures chosen for comparing toxicity are:

Average life span.

Average net gain.

Difference in net gain (control minus experimental).

Average food consumption.

Difference in food consumption (control minus experimental).

Because all feeding series were not of the same duration, it was necessary to fix the final day of experimentation from which to take the data. The 140th day was chosen as unity for the average life span. Many feeding trials terminated near the end of the 140th day. Nearly all deaths due to selenium poisoning occurred before this day.

The other data were taken during the first 70 days of experimentation. This period is satisfactory because the growth rate of control animals is rapid and apparently is not changed by a few days' difference in age. A longer period might have been chosen but the difference in growth rate is most apparent during the period of rapid growth. When feeding sub-lethal seleniferous diets, growth may be prolonged so that the experimental group approaches the control group in weight after the controls have ceased to grow.

All the data from which the comparison of toxicity was made are given in Table III. The 38 different seleniferous diets are arranged in order of decreasing selenium content. All the data for a given diet are in the row in which the selenium content is given. The age of the sample given in column 3 was the interval of time between maturity of the grain and the date the feeding began. The date of maturity for the small grains was arbitrarily set as July 15, and for the corn, October 15. Time was reckoned from the 15th day of each month.

In columns 11 to 15 inclusive are included the percentages of the experimental animals dead by the 30th, 50th, 70th, 100th, and 140th day of experimentation.

The average net gain (column 17) is the difference between the average of the weights of the experimental animals at the beginning of the series and the average at the end of the 70th day. Where there were deaths, the weight at death was taken as the weight of that animal at the end of its experimental period. In many series there was not the same number of males and females, hence it was necessary to average the males and females separately; otherwise the results would have been in error because males grow much faster than females.

Column 18 gives the difference in gain between the control and experimental groups obtained by the separate average of the males and females. The gain of the control groups for the 70-day periods is the sum of the values in columns 17 and 18.

Column 19 gives the daily food consumption of the experimental groups in grams per rat per day (total grams eaten/the total rat days). The difference in food consumption between the control and experimental animals is shown in column 20. The average daily consumption of selenium is given in column 21. This value, given in gamma, is the product of the average daily food consumption and the p.p.m. of selenium in the diet.

Values of particular interest are shown in columns 22 and 23. The percentages (based on control groups) of food eaten by the rats on seleniferous diets are shown in column 22 and the percentages of gain in weight are shown in column 23. Where there was a net loss in weight the space in this column is left blank.

Columns 24, 25, 26, 27, and 28 give ranks of toxicities obtained by ranking the individual measures of toxicity shown in columns 16, 17, 18, 19 and 20 respectively. The rank of toxicity as determined by each individual measure of toxicity was found in the following manner. The diet which killed the rats in the shortest period (column 16) was considered the most toxic by measure of toxicity 1. It was therefore ranked 1 in column 24. The diet which killed the rats in the next shortest period would accordingly receive a rank of 2 in column 24. The diet on which the rats gained the least (in many cases there was a net loss) showed that this diet was the most toxic by measure of toxicity 2, wherefore it received a rank of 1 in column 25. The diet on which there was the greatest difference in gain between the control and experimental rats indicated that it was most toxic by measure of toxicity 3. It therefore received a rank of 1 in column 26. The diet which caused the greatest voluntary restriction of food intake was the most toxic by measure of toxicity 4. It received a rank of 1 in column 27. The most toxic diet as measured by the difference in food consumption (measure 5) is the one with the largest value in column 20. This diet would then have a rank of 1 in column 28. These ranks are plotted in Figure 1.

The sums of the five ranks of toxicity for each diet are given in column 29. These sums are probably the most valuable values in the table to determine relative toxicity because they make possible a "summated" comparison of toxicity of diets. By this treatment of the data the diet with the smallest sum (column 29) is the most toxic so it ranked 1 in column 30. In two cases the sum of the five ranks of toxicity was the same. Our basis of comparison indicated the diets equally toxic, so each was given the same rank in column 30. This made it necessary to delete the next number.

In column 31 the diets are ranked from 1 to 38, in order of decreasing selenium content. In the cases where there were two, or three, diets with the same selenium content, they were given the same rank in column 31. When there were two diets with the same selenium content the following number was omitted, and where three diets had the same selenium content the middle number was used. Because there were 38 seleniferous diets to compare, it was desirable to have numbers from 1 to 38 in columns 30 and 31.

It is realized that the deletion of numbers in columns 30 and 31 may introduce a slight error. It would not be expected that the numbers in column 29, which are the sums of the ranks of five bio-assay measures of toxicity, would be identical in all cases, even if the toxicity were proportional to the selenium content. Results from animal feeding are rarely that uniform. Suppose diet No. 3 had a

RANK OF INDIVIDUAL MEASURES OF TOXICITY

Source of Selenium

ppm Se in Diet

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38

RANK OF SELENIUM CONTENT

○ Average Life Span
● Average Net Gain
● Difference in Gain (Control-Expt'l)
● Average Food Consumption
● Difference in Food Consumption (Control-Expt'l)
● More Than One Measure

Figure 1. Scatter diagram showing the ranks of the individual measures of toxicity, plotted against the rank of selenium content. A rank of 1 indicates that the diet was found to be the most toxic by the measure of toxicity. The nearer the ranks of toxicity clump to the X axis, the more toxic is the diet. "Speitz" should be replaced by "emmer" in Figure 1 and Table III.

errors due to this method of comparison would be small. In no case would the difference between the values in the same rows in columns 30 and 31, due to our method of ranking, be greater than ± 1 .

In column 32, d , which is the difference between the values in columns 31 and 30, shows the degree of relationship between the

selenium content and toxicity. If d is negative, the toxicity is less, and if positive, the toxicity is more than it would be if toxicity and selenium content were strictly proportional.

In eight cases there were no control groups, hence no values for columns 18 and 20 could be obtained from the feeding records. In order to use these series in the comparison of toxicity of the diets, arbitrary ranks were given in columns 26 and 28. The ranks given in these columns are the same as those in column 31. These are the ranks that would be in these columns if these two measures of toxicity (difference in growth and food consumption between the control and experimental) were in direct proportion to the selenium content in all

TABLE III
SUMMARY OF FEEDING SELENIFEROUS DIETS

Diet Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Selenium content p.p.m.*	Source of Selenium	Age of sample	Series Number	Date of series	Diet fed	Number of rats		Average age in days		Per cent dead by day of experimentation				
			Yr. Mo.				M	F	M	F	30	50	70	100	140
1	51.2	Na ₂ SeO ₄ †		97	8-1-34	III	5	4	28	29.5	100				
2	37.2	Na ₂ SeO ₄		96	12-12-33	III	3	5	29	29	37.5	87.5	100		
3	37.2	Na ₂ SeO ₄		107	24-9-34	III	5	5	29	28	50	50	80	100	
4	33.5	Na ₂ SeO ₄		97	8-1-34	III	5	4	28	29	55.6	66.7	86.7	77.8	88.9
5	25.0	Na ₂ SeO ₄		96	12-12-33	III	3	5	29	29	50	100			
6	25.0	Na ₂ SeO ₄		107	24-9-34	III	5	5	29	29	0	10	50	100	
7	24.6	582 Wheat	1	66	18-8-32	II	5	5	29	29	50	100			
8	24.6	582 Wheat	9	85	4-5-33	II	5	5	30	31	40	90	100		
9	23.0	570 Corn	8	62	26-6-32	II	5	5	29	29	30	90	100		
10	22.3	Na ₂ SeO ₄		97	8-1-34	III	5	4	28	29	0	0	22.2		33.3
11	22.1	607 Wheat	6	81	8-2-33	II	6	4	30	30	40	60	80	100	
12	22.1	583 Wheat	1	66	18-8-32	II	5	5	28	28	20	80	100		
13	21.3	523 Corn	1	40	4-11-31	II	2	3			60	100			
14	20.5	665 Corn	1	94	14-11-33	III	5	5	29	29	40	90	100		
15	20.5	459 Wheat	1-6	47	30-1-32	II	6	4	29	28	20	100			
16	20.5	459 Wheat	4-9	121	2-5-35	III	4	6	28.5	29.8	0	10	30	40	60
17	20.4	715 Bran‡	4-8	119	27-3-35	spe.	4	6	28.5	28.3	0	10	30	50	70
18	20.1	714 Shorts‡	4-9	121	2-5-35	spe.	4	6	28.5	29.8	0	20	30	40	60
19	18.9	586 Barley	1	68	9-9-32	II	4	3	29	29	0	14.3	42.9	100	
20	18.0	583 Barley	1	68	9-9-32	II	4	3	29	29	0	28.6	57.1	100	
21	17.5	383 Corn	2-1	8	11-12-30	I	6	9	30.3	30	0	6.7	40	73.3	100
22	17.5	383 Corn	3	8	1-11-30	I	20	20	29.0	29	15	27.5	50	80	100
23	17.5	459 Wheat	3	9	6-11-30	I	6	9	29	28	40	46.7	80	100	
24	17.2	584 Wheat	1	66	18-8-32	II	4	6	28	28	0	40	70	80	90
25	16.4	663 Corn	1	94	14-11-33	III	5	5	29	29	0	60	70	80	90
26	14.8	712 Flour‡	4-8	119	27-3-35	III	4	6	29.2	28.3	0	0	0	0	20
27	13.9	608 Barley	3	80	16-1-33	II	2	3	28	28	0	0	0	0	0
28	13.1	664 Corn	1	94	14-11-33	III	5	5	29	29	0	0	30	50	50
29	12.3	587 Barley	1	68	9-9-32	II	4	3	29	29	0	28.6	42.9	71.4	71.4
30	12.2	594 Corn	10	88	18-8-33	III	4	6	29	29	0	10	10	20	30
31	11.9	455 Barley	5	12	15-12-30	I	9	6	28	28	0	13.3	46.7	67.0	86.7
32	10.7	592 Corn	1-5	84	13-4-33	II	6	2	30	31	0	12.5	25	37.5	50
33	9.9	568 Corn	8	62	26-6-32	II	5	5	29	29	0	20	40	40	40
34	9.1	471 Wheat	4	10	3-12-30	I	2	3	28.5	28	0	0	100		
35	9.1	472 Speltz	4	10	3-12-30	I	3	2	28.7	28	0	20	60	60	100
36	8.2	531 Barley	4	41	20-11-31	II	2	3	28.0	28.7	0	0	0	0	0
37	7.5	Na ₂ SeO ₄		103	24-7-34	III	5	5	29	29	0	0	0	0	0
38	4.9	16.4% 582	2	103a	27-7-34	III	5	5	28	28	0	0	0	0	0

* Probable error of selenium content of grains = ± 1.32 p.p.m. selenium.

† All selenium salts were added to control wheat diet No. III.

‡ Milled from 459 wheat.

TABLE III—Continued
SUMMARY OF FEEDING SELENIFEROUS DIETS

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
Individual measures of toxicity						Food eaten by expd. expt./control × 100	Weight gained expt./control × 100	Statistical study									
Average life span, days	Average net gain, grams	Diff. in gain grams control-expt.	Food eaten expt. gram per rat day	Diff. in food control-expt.	% Consumed gamma Per rat (day			Rank of individual measures of toxicity					Sum of ranks	Toxic rank of diets	Se rank of diets	Difference rank ^a (column 31-30)	Diet Number
1	2	3	4	5	6			1	2	3	4	5					
13.1	-23.6	205.1	2.75	9.87	140.8	21.8		1	1	3	2	4	11	1	1	0	1
34.3	-5.1		4.51		166.8			7	2	1	12	2	31	5	5	-3	2
44.1	-18.7	223.1	3.76	9.49	139.9	28.4		11	3	7	11	32	6	2	2	-1	3
54.2	3.7	177.8	5.73	6.89	213.2	45.0	2.0	14	14	11	17	3	59	13	3	+3	4
32.9	-7.7		3.69		92.3			4	6	5	6	5	23	2	2	+1	5
68.9	1.3	203.1	4.18	9.07	104.5	31.5		20	13	4	9	14	60	14	6	+3	6
29.1	-11.0	191.6	4.21	10.09	103.6	29.4		3	5	6	11	1	26	2	2	+5	7
33.6	-16.0		3.55		87.3	25.0		6	4	7	5	7	29	4	7	+3	8
34.4	-4.0	179.1	2.98	9.68	68.5	23.5		8	11	9	3	7	38	8	9	+1	9
91.7	71.8	109.7	7.82	4.80	174.4	62.0	39.6	26	31	23	28	28	139	30	10	-20	10
44.7	-7.2	178.7	3.88	9.86	85.7	28.2		12	7	10	8	6	43	9	11	+2	11
40.9	-4.5	185.1	4.63	9.67	102.3	32.4		10	10	7	14	9	50	10	11	+1	12
25.2	-16.5		2.69		57.3			2	3	13	1	13	32	6	13	+7	13
33.4	14.1	167.7	6.00	8.43	123.0	41.6	7.7	5	15	12	19	16	67	15	15	0	14
38.4	0.5	147.2	3.54	9.39	72.3	27.4	.03	9	12	17	4	12	54	11	15	+4	15
102.3	63.4		5.28		108.2			29	30	15	15	15	104	19	15	+1	16
96.9	54.7	115.3	6.39	6.59	130.4	49.2	32.2	27	23	24	23	20	117	23	17	-6	17
99.3	62.7	119.0	6.23	5.01	125.2	55.4	34.5	28	26	21	21	27	125	25	18	-7	18
67.9	47.1	119.7	6.99	7.01	132.1	49.9	28.2	18	22	20	26	19	105	20	19	-1	19
36.4	36.4	130.4	6.91	7.99	108.2	42.9	21.8	17	20	18	20	17	93	17	20	+3	20
82.9	15.3	96.3	4.55	5.74	79.6	44.2	13.2	25	16	20	13	23	106	21	22	+1	21
71.9	24.3		5.69		100.1			21	18	22	16	22	99	18	22	+1	22
49.1	-4.9	162.1	4.19	9.51	73.3	30.6		13	9	14	10	10	56	12	22	+10	23
68.6	34.5	125.8	8.06	5.24	138.6	56.4	30.3	19	24	19	29	25	116	22	24	+2	24
62.4	21.2	160.6	6.66	7.77	109.2	46.2	11.7	16	17	16	25	18	92	16	25	+9	25
138.1	96.4		8.53	6.59	97.5	64.4	52.0	34	33	30	24	31	152	32	26	-6	26
140	109.3	68.2	9.33	4.27	120.7	68.6	61.6	37	34	32	34	29	166	35	27	-8	27
104.4	62.9	118.7	8.68	5.75	113.7	90.2	34.6	30	27	32	30	21	131	26	28	+2	28
82.1	53.0	108.8	8.99	5.04	110.2	64.0	34.8	24	25	27	33	26	135	28	29	+1	29
122.0	61.6	114.2	10.13	2.91	128.6	77.7	35.0	33	37	25	35	33	153	33	30	-3	30
82.0	61.4	85.3	6.25	3.30	75.6	63.8	41.9	23	26	30	22	32	133	27	31	+4	31
118.4	72.0	98.4	8.72	5.57	93.3	61.0	42.3	32	32	28	31	24	147	31	32	+1	32
104.6	116.7	58.4	8.90	3.76	95.2	70.3	66.6	31	35	33	32	30	161	34	33	+1	33
57.9	27.4		5.90		53.7			15	19	34	18	34	120	24	34	+10	34
79.8	28.6		7.28		65.5			22	30	34	27	34	137	29	34	+5	35
140	127.8	14.8	11.40	0.55	94.0	94.6	89.6	37	38	36	37	36	182	36	35	0	36
140	175.1	6.2	11.18	0.91	83.9	100.0	96.6	37	38	38	36	37	186	37	37	0	37
140	173.1	8.2	11.59	-0.40	58.8	103.6	95.3	37	37	37	38	38	187	38	38	0	38

^a Probable error of selenium content of grains = ± 1.32 p.p.m. selenium.

[†] All selenium salts were added to control wheat diet No. III.

[‡] Milled from 459 wheat.

cases. It is realized that this method of assigning arbitrary ranks tends to exaggerate the relationship between the toxicity of the diets and selenium content. There will be a tendency to decrease the magnitude of the numbers in column 32.

Statistical Analysis of Data

In most cases the reader can readily interpret biological data from tables or charts. In this case, however, we feel that the data in Table III warrant further analysis to bring out significant facts, inasmuch as the values given are summaries of the results of a group of experimental animals.

Several statistical methods may be used to show the relationship between the variables in Table III. Among those used, the coefficient of correlation, r , is probably the best single means of showing covariance. It should be kept in mind that a correlation coefficient of .90 is nearly three times as certain as when $r = .60$.

Two methods of obtaining r appear applicable to the data in Table III. One determines the relationship of the variables by their rank. In this case the following equation was used:

$$(1) \quad \rho_{xy} = 1 - \frac{6\Sigma(X - Y)^2}{n(n^2 - 1)}$$

where

$$\begin{aligned} \rho_{xy} &= \text{correlation coefficient between } X \text{ and } Y, \\ X &= \text{rank in selenium content,} \\ Y &= \text{rank of toxicity,} \\ X - Y &= d(\text{column 32, Table III}), \\ \Sigma &= \text{sum.} \end{aligned}$$

The other method tells the relationship between the toxic effects and selenium content. In this case the useful form of the Bravais-Pearson coefficient of correlation (Richardson, 1935) was used:

$$(2) \quad r_{xy} = \frac{n\Sigma XY - \Sigma X\Sigma Y}{\sqrt{n\Sigma X^2 - (\Sigma X)^2} \sqrt{n\Sigma Y^2 - (\Sigma Y)^2}},$$

where

$$\begin{aligned} r_{xy} &= \text{correlation coefficient between } X \text{ and } Y, \\ X &= \text{selenium content,} \\ Y &= \text{a measure of toxicity,} \\ n &= \text{number of cases.} \end{aligned}$$

Correlation coefficients were determined for each of the measures of toxicity by formulas 1 and 2. All the data necessary for the calculation of the correlation coefficients are in Table III. The selenium content (X values) is in column 1, the rank in column 31. The column from which the measures and ranks of toxicity (Y values) were taken is given after the correlation coefficient.

Measure of toxicity	r_{xy}	Column of Y value	ρ_{xy} ¹	Column of Y value
1. Average life span	-.69±.06	16	.75±.05	24
2. Average net gain	-.74±.05	17	.78±.04	25
3. Difference in net gain (control minus experimental)	.79±.05	18	.86±.03	26
4. Average food consumption	-.70±.06	19	.80±.04	27
5. Difference in food consumption (control minus experimental)	.70±.06	20	.79±.05	28
Rank of Σ of ranks			.86±.03	30
Σ of ranks	-.80±.04	29		

¹ The arbitrary ranks given to measures of toxicity 3 and 5 were not included in the calculations for the individual measures of toxicity, neither are they included in Figure 1.

One may ask if it was necessary to use several measures of toxicity, when one may suffice. A scatter diagram of the ranks of the individual measures of toxicity (Figure 1) shows that there are wide variations in the individual measures, but that there is a definite trend. In some cases the ranks of the individual measures of toxicity of a specific sample are grouped, while in other cases they show that there is a great difference in the general response of groups of animals to the different seleniferous diets. No consistent arrangements in rank of the different measures of toxicity can be found for any group of diets containing selenium from the same source.

From a statistical consideration the two correlation coefficients, $r_{zy} = .80$, and $\rho_{zy} = .86$, for all diets, indicate a high degree of correlation between the toxicity and selenium content. Due to the number of cases, the method of setting up comparable feeding experiments, and the method of determining relative toxicity of diets, the authors believe a higher degree of relationship should exist between the selenium content and toxicity of the diets, if the selenium were equally poisonous in each of the sources of selenium.

There are several groups of diets shown in Table III containing selenium from the same source. If there is a difference in the toxicity of selenium from the different sources, further analysis of the data in Table III should demonstrate it.

The coefficients of correlation using both formulas 1 and 2 were determined for the group of diets containing sodium selenite, wheat, corn, and barley. When computing ρ_{zy} it was necessary to rerank the individual groups according to selenium content and toxicity. In computing r_{zy} the selenium content was taken as the abscissa (X) and the sum of the individual ranks of toxicity as ordinate (Y).

Number of diets	Number of experimental animals	Source of selenium	ρ_{zy}	r_{zy}
6	55	Sodium selenite	.97 $\pm$.02	.96 $\pm$.02
9	90	Wheat ¹	.98 $\pm$.01	.95 $\pm$.02
10	128	Corn	.93 $\pm$.03	.97 $\pm$.01
6	46	Barley	.72 $\pm$.13	.86 $\pm$.07
38	382	All diets	.86 $\pm$.03	.80 $\pm$.04

¹ Wheat diet in column 16 was not included.

It has now been shown that there is a higher degree of correlation between the toxicity of the diets containing selenium from the same source than there is between the toxicity and selenium content of all the diets. The selenium in some of the diets must be in a more toxic form than in other diets, otherwise the correlation coefficients for the

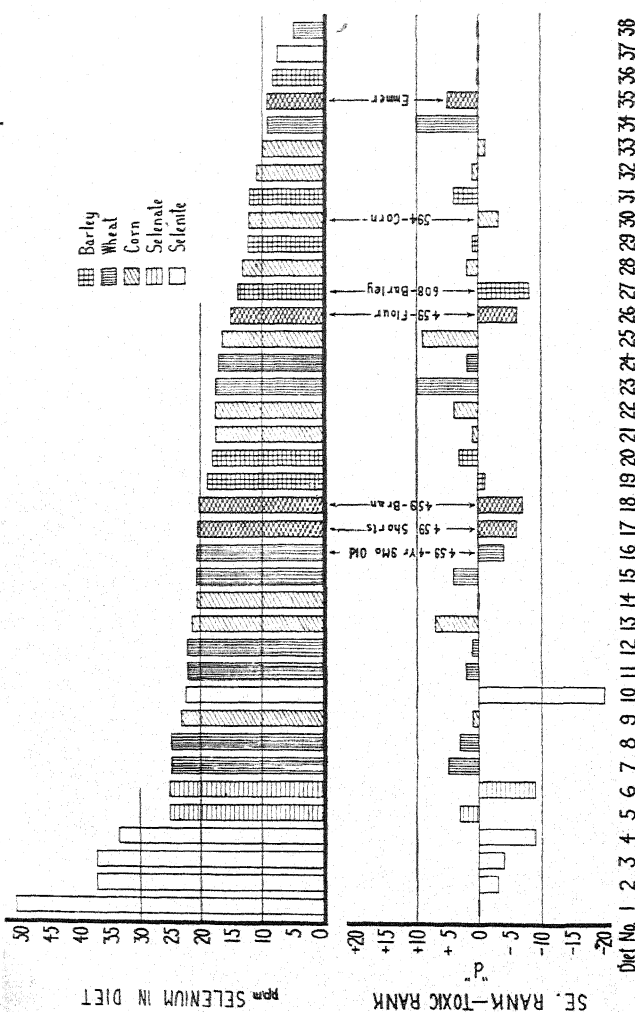


Figure 2. The difference in rank (column 32, Table III) is shown below the selenium content of the diet. If the rank of toxicity and rank of selenium content were the same, i.e., $d = 0$, all would fall on the line at zero. The sign of d indicates whether or not the diet is more or less toxic than it would be if the selenium were found to be equally toxic in all diets.

entire group of diets would have approached r for the group of diets containing selenite, corn, or wheat.

Figure 2 shows the difference in rank between the X values (selenium content) and Y values (sum of ranks of toxicity) of the diets in Table III. Diets of special interest are indicated but they will not be discussed here. The figure shows that there is some consistency in the sign and magnitude of a d for the diets containing selenium from the same source.

The differences in rank shown in Figure 2 should be of value to determine which group of diets contains selenium in the most toxic form. The sum of the differences, d (column 32, Table III), for each group of diets, was divided by the number of diets, n . The order of toxicity of selenium from the five sources is indicated in Table IV by the sign and size of the average value for each group of diets. The larger positive value indicates more toxic selenium.

TABLE IV
TOXICITY OF SELENIUM FROM DIFFERENT SOURCES

Source of selenium	Number of diets n	Number of rats	Sum of d (column 32, Table III)	$\Sigma d/n$	Order of toxicity of selenium
Wheat	9	90	+37	+4.11	1
Corn	10	128	+21	+2.10	2
Barley	6	46	-3.0	-0.50	3
Na_2SeO_4	2	18	-6.0	-3.0	4
Na_2SeO_3	6	55	-36.0	-6.0	5

In the calculation of the correlation coefficient r_{xy} , it was assumed that a linear relationship existed between the sum of the individual ranks of the measures of toxicity and the selenium content. The larger correlation coefficients were adequate proof of this relationship. The lines of regression of Y on X for each of four groups of diets are plotted in Figure 3. The best fitting line for each group of points was determined by the method of least squares.

In four cases there were two diets containing selenium at the same level from the same source. An inspection of these should indicate if our method of determining relative toxicity is dependable. The sums of the individual ranks of toxicity for each pair of diets containing selenium from the same source at the same level were: for selenite 31 and 32, for No. 582 wheat 26 and 29, and for No. 388 corn 99 and 106. These show good agreement. The sums of the ranks of toxicity for the diets containing 25 p.p.m. of selenium as sodium selenate were 26 and 60. Reference has been made to the apparent difference in toxicity of different lots of a selenium salt (Franke and Potter, 1935).

It should be recalled that the feedings of these diets containing selenium from the same source were not carried out at the same time.

The results of biological experiments are usually dependent upon a number of variables working together or independently. If we can determine to what degree other factors than selenium affected the results, it should be possible to discover if selenium is the limiting factor.

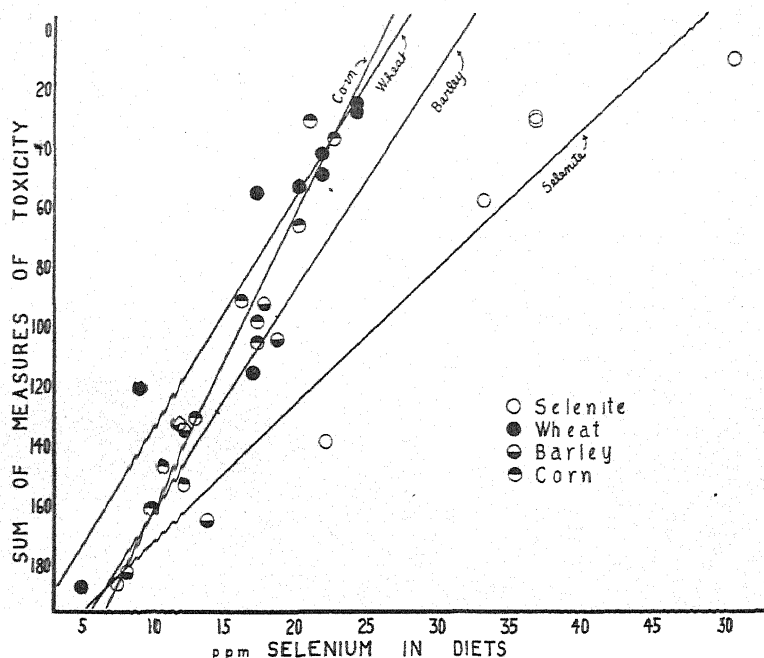


Figure 3. Regression lines showing the relative toxicity and increase in toxicity with an increase in selenium content of four groups of diets. The smaller the sum of the measures of toxicity, the more toxic is the diet.

The correlation coefficient for measure of toxicity (3) (average net gain) of .74 indicates that other variables than the difference in toxicity of selenium from the different sources reduced the magnitude of r . None of the correlation coefficients for the groups of diets containing selenium from the same source reached unity. The problem is to determine to what degree the variability of experimental conditions reduced the correlation coefficients.

The standard error of estimate, a measure of the dispersion about the regression line, is given by the equation:

$$(3) \quad S_y = \sqrt{\frac{\sum (y - \bar{y})^2}{n}}$$

where y = observed values,
 $\bar{y}$ = computed values (or regression line),
 n = number of observations.

Any regression line in Figure 3 shows the dispersion of the observed values about the regression line. In the present case the units measured on the ordinate (Y axis) would be in grams instead of the sums of the ranks of toxicity as shown in Figure 3. The standard error of estimate is a measure of the dispersion due to all other variables because when $S_y = 0$, all the points fall on the regression line and $r = 1$.

The standard deviation, σ_y , a measure of the total dispersion on the Y axis, is given by the equation:

$$(4) \quad \sigma_y = \sqrt{\frac{\sum (y - \bar{y})^2}{n}},$$

where y = observed value,
 $\bar{y}$ = mean of y .

The standard deviation is made up of two components:

- a. The dispersion due to difference in selenium content of the diets.
- b. The dispersion due to all other variables.

The control groups did not grow at the same rate as shown by the standard deviation of 22.4. If the experimental animals were subject to the same variation as the control animals, this variable should cause a dispersion about the regression line, S_y , equal to the dispersion on the Y axis. From the relation

$$(5) \quad S_y = \sqrt{S_{y \cdot 0}^2 - \sigma_{y \cdot r}^2},$$

where $S_{y \cdot 0}$ = observed standard error of estimate,
 $\sigma_{y \cdot r}$ = standard deviation of control groups,

we can determine how much closer the observed values would come to the regression line had there been no variability in experimental conditions. By equation (3) $S_{y \cdot 0} = 32.3$ and by equation (4) $\sigma_{y \cdot r} = 22.4$. These values substituted in equation (5) gave $S_y = 23.3$. If there had been no variability of experimental conditions, the standard error of estimate would have been 23.3 instead of 32.3. This value, $S_y = 23.3$, can be substituted in an equation for r_{xy} :

$$(6) \quad r_{xy} = \sqrt{1 - \frac{S_y^2}{\sigma_y^2}}.$$

where σ_y = standard deviation of experimental groups.

From equation 6, r_{xy} was found to be .88. If we are justified in making the assumption that the experimental groups were subject to the same variation as the control groups, we can say that the correlation coefficients would have been .88 instead of .74.

The prediction value of a correlation coefficient (probability of any value falling within a given distance from the regression line) is given by the relation, $1 - \sqrt{1 - r^2_{xy}}$ (Treloar, 1935). When the value of r increased from .74 to .88, the prediction value increased by 1.6.

It does not seem amiss to assume that the correlation coefficients would have higher values if it were not for the factors inherent in the experimental conditions. When the prediction value is increased 1.6 times, the correlation coefficients would approach unity for some of the diets containing selenium from the same source. When the prediction value of a correlation coefficient of .95 is increased 1.6 times, the correlation coefficient becomes approximately .98. It can be seen that some of the correlation coefficients would exceed .99.

Discussion

From the correlation coefficients for the five measures of toxicity it is most significant that for the large number of cases which are included in Table III, each shows nearly the same degree of correlation. With individual samples single measurements may vary within wide limits as Figure 1 shows. This emphasizes the necessity for several measures of toxicity to describe adequately a given sample but for the entire group of diets any single measure appears to show nearly the same degree of relationship between toxicity and selenium content.

The correlation coefficients found from the ranks of the measures of toxicity, ρ_{xy} , are slightly higher than those obtained from the recorded values obtained from the records of feeding trials, r_{xy} , probably because there is not a uniform change in the selenium content of the diets. The first four diets have high selenium contents (Figure 2) which rank does not take into account, but the toxicity of these diets is near that of some of the cereal diets.

The values of the correlation coefficients for the measure of toxicity, average life span, are lower than for the other measures of toxicity, because it was necessary to fix the final day of experimentation. All feeding trials did not continue until all animals died. There were four diets which did not kill a single rat so this measure of toxicity must record them equally toxic. The other measures of toxicity show that these four diets were not equally toxic.

That the diets containing selenium from the same source (selenate is an exception) are consistent in their rank is apparent from Figure 2.

There is not a diet containing sodium selenite with a positive sign for the value of d . The first diet (Table III) contained so much more selenium than any of the other diets (51.2 p.p.m.) that it would be expected to be the most toxic. As the selenite diets approach a selenium content comparable to the cereal diets the magnitude of d uniformly increases. The value of d for the selenite diet number 37 is 0, but the next diet containing a toxic cereal contains only two-thirds as much selenium. The two diets have approximately identical toxicities because the sums of the individual ranks of toxicity are 186 and 187.

Only one wheat diet has a negative value for d . The exception is diet number 16, in which the sample was nearly five years old when fed. All other toxic wheat diets have positive values from 1 to 10 for d except diet No. 38, in which $d = 0$. In not a single case is the sum of the five ranks of toxicity for a given wheat diet less than the sum for a wheat diet containing more selenium. This is also true of the diets containing sodium selenite.

The corn and barley diets likewise show good agreement within their groups. Only two of the ten corn diets have negative values for d (Figure 2). In three cases the toxicity was not in the same order as the selenium content (Table III) but the difference in selenium content was of about the same order of magnitude as the probable error in selenium analysis, and the sums of the individual ranks of toxicity differed only slightly. Only one barley sample (No. 608) is out of line with the rest of the barley group.

The remarkably high correlation in the groups of diets containing selenium from the same source indicates that the selenium in each kind of grain has a degree of toxicity which is specific for each kind of cereal. The toxicity of selenium in the different groups in Table IV was found to be: wheat > corn > barley > selenate > selenite.

The correlation coefficients for the corn, wheat and selenite diets suggest a perfect causal relationship between selenium content and toxicity. If we assume that there was the same variability of experimental groups as there was of control groups, and use the device of increasing the prediction value, some values of r become so near unity as to indicate that selenium is the limiting factor. The values of r for the wheat and corn diets are approximately the same as for the selenite diets in which selenium is known to be the sole toxicant. This is evidence against the presence of another toxicant, unless it is directly associated with selenium. *Until the presence of other poisons is demonstrated, it can be assumed that the toxic effects produced in experimental animals are due to selenium.* Other poisons have not been found and those suggested as possibilities were not as toxic as selenium by oral feeding (Franke and Moxon, 1937).

Table IV further shows that there is little difference in toxicity of selenium in the different cereals. The value of $\Sigma d/n$ for the group of barley diets would have been 1.16 had it not been for the diet containing barley No. 608. The position of the barley group, however, would not have been changed.

The lines of regression, Figure 3, plainly show the relative toxicity of the different groups of diets. From the point of intersection of any horizontal line with each of the regression lines one should be able to determine the selenium content necessary to have four equally toxic diets from each of the four sources. Suppose a horizontal line were drawn from the point where the sum of the ranks of toxicity is 105. The wheat diets containing 14 p.p.m. of selenium, the corn 16 p.p.m., the barley 18 p.p.m., and the selenite 25 p.p.m. would have equal toxicities. The regression lines also show the relative increase of toxicity with selenium content. The points fit the curves fairly well. The curves show that there is less difference between the toxicity of selenium in the different cereals than between the cereals and selenite.

The results are not conclusive proof that selenium in wheat is more toxic than selenium in corn or barley as is shown in Table IV. Possibly a similar slight difference in observed toxicity would have resulted if selenium salts had been added to a barley or corn diet. Our feeding trials indicate on the other hand that in such cases a difference in observed toxicity would not be probable. The correlation coefficient r_{xy} for all diets containing the cereals grown in 1932 is +.94. These included five wheat, six corn, and four barley diets. The coefficient .94 shows about as high a degree of relationship as was found for the diets containing selenium from the same source. It has been shown (Painter and Franke, 1935) that the selenium in the cereals is in organic compounds. It is doubtful if the cereals would synthesize different compounds of selenium; however, the relative proportion of the compounds may vary in the different grains.

The slope of the regression line for the corn diets is greater than the slope for the other cereal diets (Figure 3). With increased selenium content in the corn diets there is a more rapid increase in toxicity. The curves for wheat and corn intersect at about 23.0 p.p.m. The curves indicate that above this level corn diets would be more toxic than wheat diets containing equal amounts of selenium. Three corn diets with selenium contents near the extreme levels have greatly affected the slope of the curve.

Only eight of the thirty diets containing selenium as it occurs in seed grains have negative values in column 32, Table III, or Figure 2. Two of these are diets containing corn sample No. 594 and barley sample No. 608. These appear to have the least toxic selenium in their

respective kinds of grain. These two samples were grown the same year in the same field. The other sample grown in this field at the same time, No. 607 wheat, shows a toxicity consistent with the other wheat samples.

Four of the cereal diets of low toxicity contain wheat No. 459 and its milled fractions. Sample No. 459 was fed several different times. The results are given in tabular form to show the apparent change in toxicity. It is probable that the value of d for diet No. 16 would have been a larger negative number had not arbitrary values been given ranks of measures of toxicity 3 and 5. It is evident that this sample showed a decrease in toxicity as it aged.

Diet number	Sample number	Age of sample when fed	Value of d^2
23	459 Wheat	3 months	+10
15	" "	1 year, 6 months	+4
16	" "	4 years, 9 months	-4
26	712 Flour ¹	4 years, 8 months	-6
17	714 Bran ¹	4 years, 8 months	-6
18	715 Shorts ¹	4 years, 9 months	-7

¹ Milled from 459 wheat a short time before fed.

² Average value of d for wheat = -4.11.

The results with the milled products show that the selenium in the bran, flour and shorts is equally toxic. Horn, Nelson and Jones (1936) found that the "toxic principle" was quite uniformly distributed in the milled fractions of wheat.

Franke and Potter (1935) suggest that some of the selenium in selenite may be reduced to metallic form before it is absorbed. They found metallic selenium non-toxic and selenide slightly toxic. Selenates are much more difficult to reduce than selenites and selenides oxidize to metallic selenium readily in air. The toxicity of selenium from all the sources fed is in the order: wheat > corn > barley > selenate > selenite > selenide > metallic selenium. Based on the degree of inhibition of CO₂ production by yeast cells, Moxon and Franke (1935) found the above order of toxicity of selenate and selenite reversed. The selenite was very toxic and the selenate virtually non-toxic. From injecting selenium salts into albino rats, Franke and Moxon (1936) found the minimal fatal dose for selenite less than for selenate. The mechanism of selenium toxicity in all its forms can hardly be attributed to its reduction. It is probable that selenium as it occurs naturally in cereals is not in a high state of oxidation.

The amount of selenium consumed in gamma per rat per day, shown in column 21, does not appear to correlate with the other data. In some cases relatively large quantities of selenium were consumed

without causing a large percentage of deaths in the animals. The rats on the diets containing selenium salts ate more selenium than those fed selenium in the toxic grains. It might be suspected that the toxic symptoms observed in rats would be a direct function of the quantity of selenium consumed per day. By ranking the values in column 21 and determining the difference between this rank and the rank of toxicity of the diets no relationship was found between the selenium consumed and the observed toxicity. The correlation coefficient ρ_{xy} as found by formula 1 had a value of .03 which proves that virtually no relationship exists. Rats on diets containing 10 to 15 p.p.m. of naturally occurring selenium ate more selenium per day than the rats on diets containing about 25 p.p.m. of naturally occurring selenium. Although there is a high correlation between the selenium content of the diet and voluntary food restriction, there is no correlation between the toxic effects produced and the daily consumption of selenium. In selenium poisoning there is a pronounced restriction of food consumption which is not great enough to cause death by starvation in control rats (Franke and Potter, 1935) but the food intake is reduced to a level which will not maintain life and permit the animal to withstand the poisonous action of selenium. In general, when there was a tendency toward voluntary food restriction, the rats eating the most selenium fared the best. This can be partly explained by the fact that those animals more resistant to selenium poisoning ate more as they became older. Some rats on lower levels of selenium grow so they ingest larger quantities of selenium as their body weights increase.

It has been shown (Franke, 1934; Franke and Potter, 1935, 1936) that animals fed seleniferous diets *ad lib.* voluntarily restrict their food consumption. The actual food consumption (based on percentage of control) is shown for the seleniferous diets in column 22. In all cases excepting the two diets with the lowest selenium content, the experimental rats ate less than the control rats. When the diets contained more than 10 p.p.m. of selenium, the food consumption was reduced approximately 30%. With some diets containing extremely toxic concentrations, the restriction of food reached nearly 80%.

It may be believed that the restriction of food consumption is entirely responsible for the retarded growth rate of animals fed diets containing small amounts of selenium. The values in column 23 show that in every case the percentage of gain of the experimental animals is less than the percentage eaten. There can be no question that the retarded growth caused by small amounts of selenium is not entirely due to reduced food intake. Since animals receiving seleniferous diets voluntarily restrict their food consumption, a greater proportion of the total food consumed is necessary for maintenance. No doubt this is

partly responsible for the observed results. The fact that the animals on a seleniferous diet gain less per gram of consumed food is of tremendous economic importance to the farmer feeding seleniferous grains. He cannot obtain the net unit gain that he could if he fed a "normal" grain.

The minimum concentration of selenium that may be harmful has not yet been found. It is safe to predict that any diet containing over 5 p.p.m. of selenium will retard the growth of young animals. Relatively small quantities of selenium in the diet will produce death. All diets but one shown in Table III containing more than 9.1 p.p.m. of selenium caused deaths in young animals. These conclusions are based on studies on the rat. Recently Smith, Stohlman, and Lillie (1937) have shown the rat to be more resistant to selenium poisoning than the cat or rabbit. Little is known about the relative susceptibility of farm animals to the poisonous action of selenium.

Summary and Conclusions

The results of feeding 38 seleniferous diets to albino rats have been summarized. Three hundred and eighty-two experimental animals were used in these feeding trials. In most of the trials the experimental group was paralleled by a control group. The sources of selenium were sodium selenite, sodium selenate and naturally occurring selenium in wheat, corn, barley and emmer. The rank of toxicity and relative toxicity of the diets have been determined and the toxicity compared with the selenium content.

Correlation coefficients were computed for all diets, and for the diet groups containing selenium from the same source. A high degree of correlation was found between the toxicity and selenium content. The correlation coefficients for the groups of diets containing selenium from the same source were usually larger than for the entire group of diets. It was shown that the selenium from the different sources varied in toxicity. If selenium is the sole toxicant in the cereals fed, the relative toxicity of selenium in the different diets is in the order: wheat > corn > barley > selenate > selenite.

If the experimental animals were subject to the same variability as the control animals, and this is taken into account in the calculation of the correlation coefficients, some values of r become so near unity that it can be assumed that selenium is the limiting factor.

There was a pronounced restriction of food consumption in every diet containing over 10 p.p.m. of selenium as it occurs in the cereals.

The percentage of gain in the experimental animals was less than the percentage of food consumption (percentage based on control

groups. This means that the gain per gram of diet consumed was less for the seleniferous diets than for control diets.

Concentrations of less than 5 p.p.m. of selenium in diets will prevent normal growth. In every case shown in Table III the rats on seleniferous diets gained less than the control rats.

All but one of 35 diets containing over 9.0 p.p.m. of selenium caused deaths in young animals. It is probable that only naturally occurring selenium will regularly cause deaths at this level.

Female rats are slightly more susceptible to selenium poisoning than males.

There was no relationship between the absolute amount of selenium consumed per day and observed toxic effects. The effect of seleniferous diets appears to depend more on the concentration of selenium in the diet than upon the quantity of selenium consumed per day.

Evidence is presented that a toxic grain decreased in toxicity after it had been stored several years.

If the results obtained by feeding rats can be applied to larger animals, it is evident that farmers have suffered great losses by feeding seleniferous feeds with surprisingly low selenium contents.

Literature Cited

- Beath, O. A., Draize, J. H., and Gilbert, C. S.
1934 Plants poisonous to livestock. Wyo. Agr. Exp. Sta. Bull. 200, p. 82.
—, —, Eppson, H. F., Gilbert, C. S., and McCreary, O. C.
1934 Certain poisonous plants of Wyoming activated by selenium and their association with respect to soil types. J. Am. Pharm. Assoc. 23: 94-97.
—, Eppson, H. F., and Gilbert, C. S.
1935 Selenium and other toxic minerals in soils and vegetation. Wyo. Agr. Exp. Sta. Bull. 206, p. 55.
Byers, H. G.
1934 Selenium, vanadium, chromium, and arsenic in one soil. Ind. Eng. Chem., New Ed. 12: 122.
1935 Selenium occurrence in certain soils in the United States, with a discussion of related topics. U. S. Dept. Agr. Tech. Bull. 482, p. 47.
Draize, J. H., and Beath, O. A.
1935 Observations on the pathology of blind staggers and alkali disease. J. Am. Vet. Med. Assoc. 86: 753-763.
Franke, K. W.
1934 A new toxicant occurring naturally in certain samples of plant foodstuffs. I. Results obtained in preliminary feeding trials. J. Nutrition 8: 597-608.
1934a A new toxicant occurring naturally in certain samples of plant foodstuffs. II. The occurrence of the toxicant in the protein fraction. *Ibid.* 8: 609-613.
1935 A new toxicant occurring naturally in certain samples of plant foodstuffs. X. The effect of feeding toxic foodstuffs in varying amounts, and for different time periods. *Ibid.* 10: 223-231.
— and Moxon, A. L.
1936 A new toxicant occurring naturally in certain samples of plant foodstuffs. XV. A comparison of the minimal fatal doses of selenium, tellurium, arsenic, and vanadium. J. Pharmacol. and Exper. Therap. 58: 454-459.
1937 The toxicity of orally ingested arsenic, selenium, tellurium, vanadium and molybdenum. *Ibid.* 61: 89-102.

- , Poley, W. E., and Tully, W. C.
 1936 A new toxicant occurring naturally in certain samples of plant foodstuffs. XII. Monstrosities produced by the injection of selenium salts into hens' eggs. *Anat. Record* **65**: 15-24.
- and Painter, E. P.
 1935 Selenium in proteins from toxic foodstuffs. IV. The effect of feeding toxic proteins, toxic protein hydrolysates, and toxic protein hydrolysates from which the selenium has been removed. *J. Nutrition* **10**: 599-611.
- 1936 Selenium in proteins from toxic foodstuffs. I. Remarks on the occurrence and nature of the selenium present in a number of foodstuffs or their derived products. *Cereal Chem.* **13**: 67-70.
- and Potter, V. R.
 1934 A new toxicant occurring naturally in certain samples of plant foodstuffs. III. Hemoglobin levels observed in white rats which were fed toxic wheat. *J. Nutrition* **8**: 615-624.
- 1935 A new toxicant occurring naturally in certain samples of plant foodstuffs. IX. Toxic effects of orally ingested selenium. *Ibid.* **10**: 213-221.
- 1936 A new toxicant occurring naturally in certain samples of plant foodstuffs. XIII. The ability of rats to discriminate between diets of varying degrees of toxicity. *Science* **83**: 330-332.
- 1936a A new toxicant occurring naturally in certain samples of plant foodstuffs. XIV. The effect of selenium containing foodstuffs on growth and reproduction of rats at various ages. *J. Nutrition* **12**: 205-214.
- and Tully, W. C.
 1935 A new toxicant occurring naturally in certain samples of plant foodstuffs. V. Low hatchability due to deformities in chicks. *Poultry Sci.* **14**: 273-279.
- 1936 A new toxicant occurring naturally in certain samples of plant foodstuffs. VII. Low hatchability due to deformities in chicks produced from eggs obtained from chickens of known history. *Ibid.* **15**: 316-318.
- Horn, M. J., Nelson, E. M., and Jones, D. Breese
 1936 Studies on toxic wheat grown on soils containing selenium. *Cereal Chem.* **13**: 126-139.
- Moxon, A. L., and Franke, K. W.
 1935 A new toxicant occurring naturally in certain samples of plant foodstuffs. VIII. The effect of certain salts on enzyme activity. *Ind. Eng. Chem.* **27**: 77-81.
- Painter, E. P., and Franke, K. W.
 1935 Selenium in proteins from toxic foodstuffs. III. The removal of selenium from toxic protein hydrolysates. *J. Biol. Chem.* **111**: 643-651.
- Richardson, C. H.
 1935 Introduction to statistical analysis. Harcourt, Brace and Company, New York.
- Robinson, W. O.
 1933 Determination of selenium in wheat and soils. *J. Assoc. Official Agr. Chem.* **16**: 423-424.
- , Dudley, H. C., Williams, K. T., and Byers, H. G.
 1934 Determination of selenium and arsenic by distillation. *Ind. Eng. Chem. Anal. Ed.* **6**: 274-276.
- Schneider, H. A.
 1936 Selenium in nutrition. *Science* **83**: 32-34.
- Smith, M. J., Stohman, E. F., and Lillie, R. D.
 1937 The toxicity and pathology of selenium. *J. Pharmacol. and Exper. Therap.* **60**: 449-472.
- Steyn, D. G.
 1934 The toxicology of plants in South Africa. *J. Vet. Sci. and Animal Ind.* **3**: 359-473.
- Treloar, A. E.
 1935 An outline of biometric analysis. Burgess Publishing Company, Minneapolis, Minnesota.

IMBIBITION OF WATER BY PROTEINS

DOROTHY JORDAN LLOYD

British Leather Manufacturers' Research Association, London, England

Read at the Annual Meeting, May 1937)

Proteins, as they occur in nature, always occur in association with water. Dry proteins are purely artificial products and special precautions have to be taken in their preparation. Moreover, the dividing line between a protein which has lost water till it is dry and one which has lost water till it has begun to change its character is not always very well defined.

Proteins are hygroscopic bodies and dry proteins placed in contact with water as vapor or as liquid absorb it with evolution of heat and loss of total volume. The amount of water absorbed depends among other things on the activity or effective concentration of the water available. The equilibrium between water and protein is reversible. Proteins in equilibrium with water at high activity values will give up water, if the activity of the water in the system is brought to low values. The curve connecting the activity of the water with the amount held by the protein is not, however, a simple, smooth curve. The water appears to go in or out of the system in two stages and over a wide range is independent of the activity.

Katz (1917) studied the amount of water held by casein and other proteins in atmospheres of varying degrees of saturation. In atmospheres more than half saturated (that is, with activity values > 0.5), the amount held was a function of the activity; in atmospheres one-half to one-fifth saturated (activities 0.5 to 0.2), the amount held was practically constant, while in atmospheres less than one-fifth saturated, the amount again fell with decreasing saturation.

Water can be drawn out of moistened proteins by other means than evaporation. Freezing is an effective method and has been used by Moran (1926, 1932, 1935). Moran has measured the amount of water held by a number of proteins against the temperature at which ice crystals begin to form, that is, the temperature at which the forces binding water to water in the crystal lattice of ice, become greater than the forces binding water to protein. He also finds that the water held in protein systems can be considered under two headings—loosely bound water, the amount of which depends on the temperature but which is removed by freezing to -20° , and firmly bound water which resists freezing apparently to temperatures as low as -100° .

Water can also be expelled from protein systems by direct pressure, a method used by Jordan Lloyd and Moran (1934) for studying the relations of water in iso-electric gelatin gels. Here again the same relation is found; the water in the gel comes out in two stages. The amount of loosely bound water held in the gel is a function of pressure,

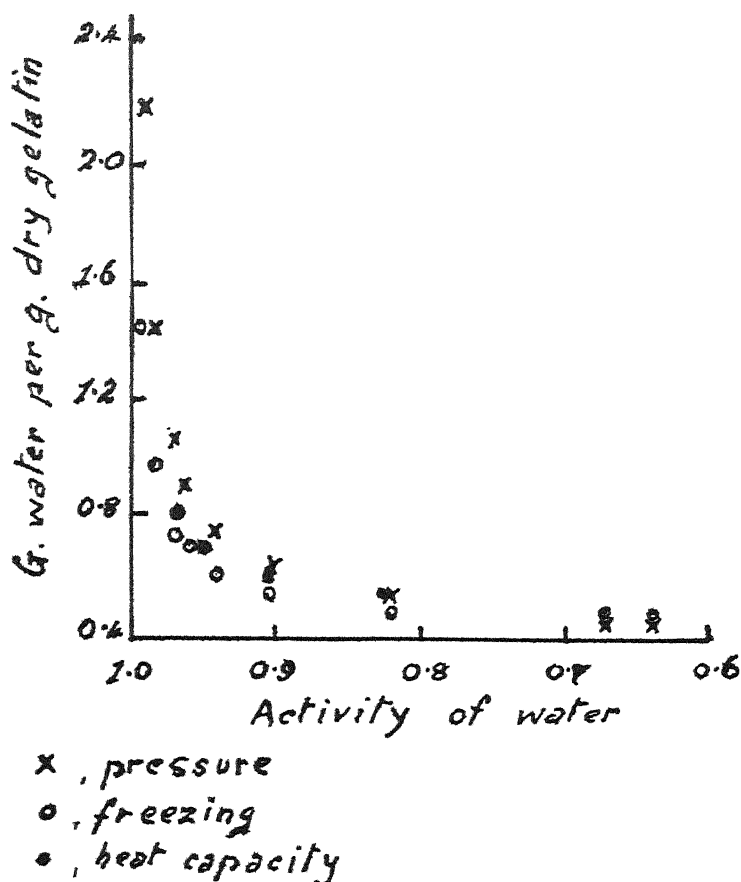


Figure 1.

falling with increasing pressure, but apparently completely removed by a pressure of 7,000 pounds per square inch. At pressures from 7,000 to about 30,000 pounds per square inch, the amount of water held is very nearly constant, but at higher pressures, still more water, namely, the firmly bound water, begins to leave the system.

The variation of the activity of water with vapor pressure, temperature and pressure can be calculated. If the amount of water

held per gram of dry protein is plotted against the activity of the water, all three methods give remarkably consistent results. The bound water can also be measured by calorimetric methods (Hampton and Mennie, 1932). The results obtained for iso-electric gelatin by pressure, freezing, and heat capacity measurements are shown in

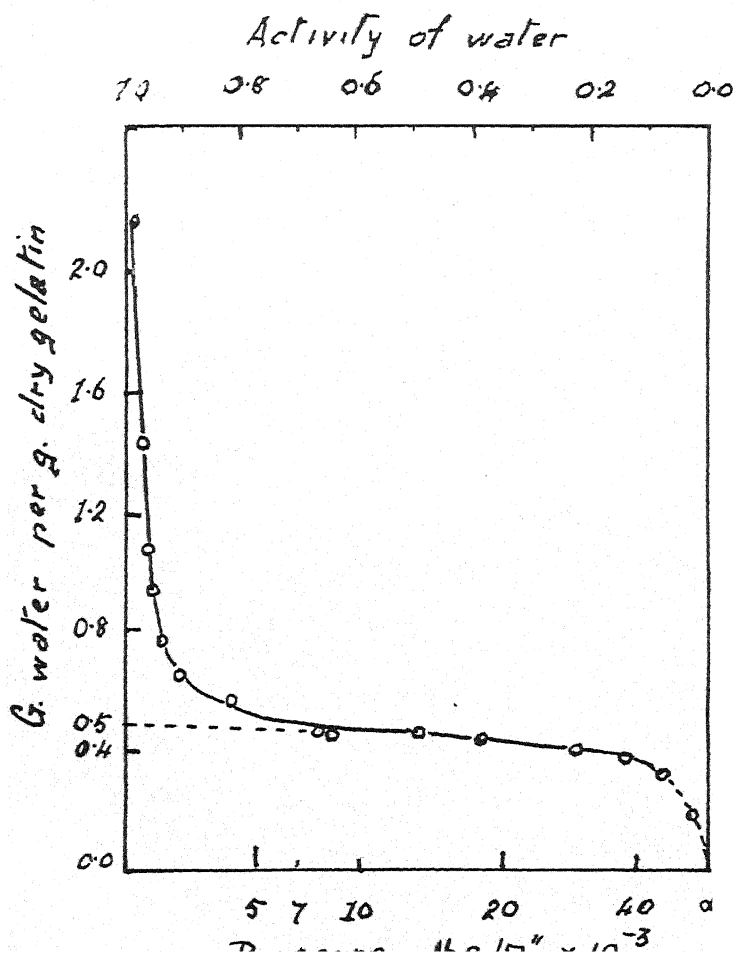


Figure 2.

Figure 1. This figure only shows bound water over the range of activities from 1.0 to 0.6. Figure 2 shows the bound water plotted against pressure for gelatin over the full range of activity. This shows very clearly that for a wide range of activities, the bound water of gelatin is constant at 0.5 gram per gram of dry gelatin.

The activity of water at which the loosely bound water is given up appears to be much the same for a number of proteins. This is shown in Figure 3, which illustrates results obtained by Moran (1935) in

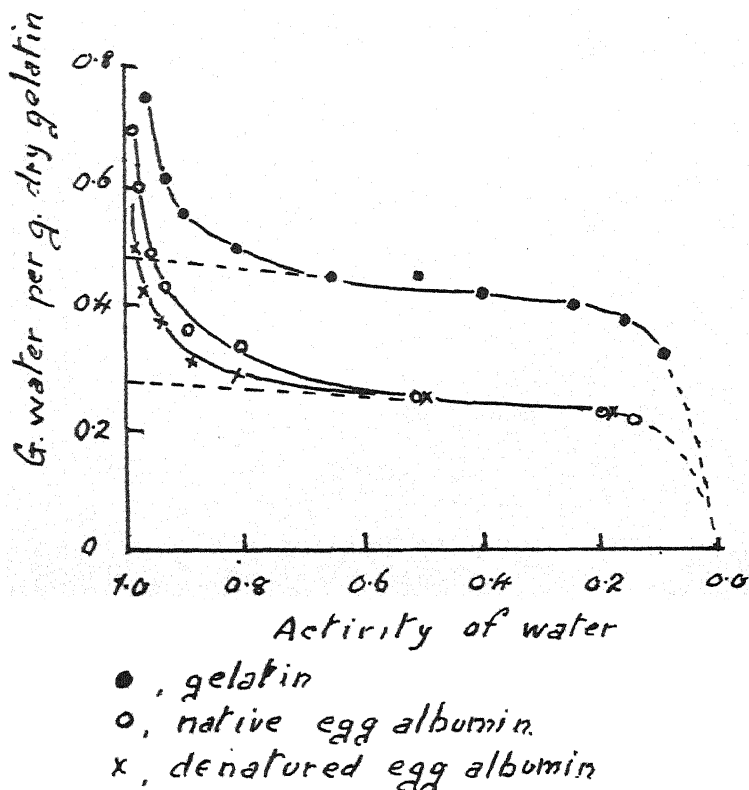


Figure 3.

freezing experiments with gelatin, native egg albumin and denatured egg albumin. The actual amount of water bound differs in the different proteins. Moran (1935) gives the following figures for firmly bound, or, as he now calls it, chemically bound water:

Gelatin, 0.5 g. water per 1 g. dry protein.

Native egg albumin, 0.26 g. water per 1 g. dry protein.

Denatured egg albumin, 0.26 g. water per 1 g. dry protein.

Myogen, 0.4 g. water per 1 g. dry protein (approximately).

Total solids of muscle, 0.33 g. (0.15 g., Brooks, 1934) water per 1 g. dry protein.

The amount of loosely bound water also differs in different proteins. Moran's experimental figures suggest that there is less loosely bound

water in denatured egg albumin than in native egg albumin, and that in myogen there is a comparatively small amount at all activities of water.

The evidence on the whole seems convincing that water is held to protein molecules by two different methods, one of which forms a much closer association than the other. There has been considerable discussion as how best to define "bound water." Probably the best definition is that it is that part of the water in a system which is no longer available for dissolving other solutes. This definition arises largely from the work of Gortner and his colleagues which has done so much toward showing both the theoretical and the practical importance of this aspect of protein science (Newton and Gortner, 1922, and Jones and Gortner, 1932). Gortner and his colleagues added a known amount of sucrose to expressed plant juices, and measured the resulting depression of the freezing point which was found to be greater than the value calculated on the assumption that all the water present was free to dissolve the sucrose. The obvious inference is that all of the water is not free, but that some is bound to the proteins present in the expressed juices. It should, perhaps, be mentioned here that Greenberg and Greenberg (1933) have added sucrose to protein solutions and then submitted them to ultra-filtration and state that the protein does not retain more than 10% of its own weight of water under these conditions. However, even if opinions differ as to the amount of bound water, and even if there is not as yet a generally accepted definition of bound water, there is no doubt that proteins can and do hold water in a very close association. A direct demonstration of this has been made by Hatschek (1936) who showed that a gelatin gel containing cobalt chloride showed the characteristic pink color of the hydrated cobalt salt, but that if it were allowed to dry slowly by evaporation, it turned to the blue color of the anhydrous cobalt salt, while there was still 33% of water in the gel.

Even though there is still much investigation needed, it may not be without profit to consider the possible forms which an association between water and protein might take.

In the first place, reference must be made to the work of Cohn and his colleagues at Harvard on the influence of proteins on the dielectric properties of water (see especially Cohn, 1936; Wyman, 1936). This, more than any other recent work, has led to the vivid realization of the protein molecule as a multipolar particle. On account of this multipolar structure, the molecule will be surrounded by complicated fields of force between the positive and negative poles (the so-called Coulomb forces), and the polar molecules of water will become orientated in the neighborhood of a protein molecule according to the strength and direction of these forces.

The strength of the Coulomb forces around a protein molecule will depend not only on the number of polar pairs, but also on their arrangement over the surface. Anything which leads to an alteration in their number or configuration will lead to an alteration in the number of polar molecules of water held around the protein molecule. Moran's (1935) recent work shows that denaturation leads to a reduction in the amount of loosely bound water, and Astbury has shown that denaturation is an alteration in the configuration of the molecule causing the open globular form of the water soluble protein to become more fibrous and compact (see especially Astbury and Lomax, 1935; Astbury, Dickinson, and Bailey, 1935). This might well lead to the positive and negative poles of the protein molecule being brought closer together with an accompanying reduction of the polar moment, and release of some of the bound water (see Cohn, McMeekin, Edsall, and Blanchard, 1933).

The amount of water held around a protein molecule by these interionic forces must obviously be affected by the nature, number, and distribution of any other ion pairs present. Moran has shown that the presence of salts reduces the amount of loosely bound water, a result which would be expected if the molecules are held in the polar fields around all the ions present in the solution.

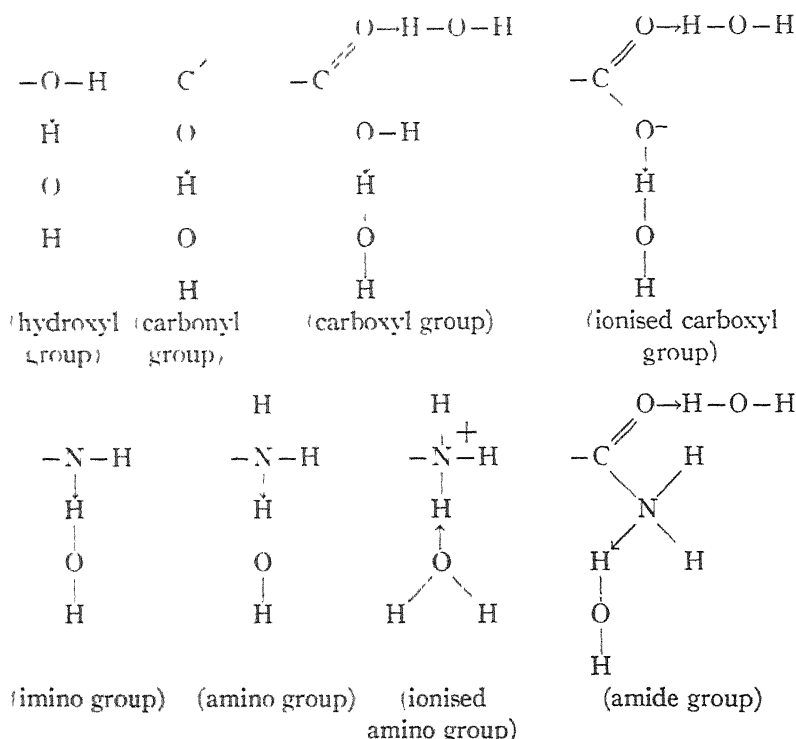
The effect of adding acids or alkalies to a protein system is interesting. It is well known that proteins are most easily precipitated by dehydrating agents, such as alcohol, at their iso-electric points, and Hardy, as long ago as 1905, suggested that electrically charged proteins were more heavily hydrated than those which are iso-electric to the surrounding medium. The addition of acid or alkali to a protein, by suppressing one member of a zwitterion pair, will undoubtedly alter the polar configuration of the protein molecule. Moreover, the prevailing charged centers of the protein molecule (positive in acid solutions and negative in alkaline ones) will now have as their gegenions comparatively small ions of possibly the same or possibly a different valency. This in itself will lead to alteration in the localization of the water around the protein molecule, and if Fajan's principle holds, would lead to an increase in the amount of loosely bound water, the increase being greater for monobasic than for dibasic acids, which appears to be the case (see Jordan Lloyd and Phillips, 1933).

The balance of evidence with regard to the multipolar character of the protein zwitterions suggests therefore that this multipolar character is sufficient to account for the loosely bound water of proteins—water which is not a fixed quantity per molecule of protein, but varies according to the general distribution of the interionic forces throughout the system.

When we come to consider the firmly bound water, the matter assumes a somewhat different complexion. To begin with, although the evidence is scanty, what there is suggests that it is a much more constant quantity. Moran's (1926) experiments carried out by the freezing method suggest that the amount of the closely bound water is not altered by denaturing the protein. Moreover, other experiments suggest that it is not altered by the presence of salts (Moran, 1932). Some experiments carried out by my colleague, Dr. F. E. Humphries, give a figure of 0.46 g. of bound water per gram of dry gelatin at pH 2.6 compared with 0.48 g. in the iso-electric state. The water was removed by pressure and the figures indicate that the addition of acid to the gelatin has not altered the amount of closely bound water. The inference is, therefore, that neither altering the architectural pattern of the protein molecule nor altering the interionic forces throughout the system appreciably affects the closely bound water. A consideration of the constitution of the protein molecule shows that it must possess the capacity to coordinate with water molecules. For instance, where any of the side chains of the molecule contain oxygen or nitrogen atoms, there will be possibilities of co-ordination with water, and a protein containing a number of such side chains will have a greater affinity for water than a protein in which the side chains are mostly hydrocarbon chains. There are also oxygen and nitrogen atoms at every peptide link down the main polypeptide backbone, and the capacity to coordinate with water will be greater in protein systems where the molecules exist in the free state than in systems like fibers where they are held together in bundles by direct carbonyl-imino links between adjacent polypeptide chains.

The oxygen atoms of protein molecules occur in carbonyl, amide, or hydroxy groups, the nitrogen atoms in amide, imino, or amino groups. In all these groups the oxygen and nitrogen atoms possess electrons which are not utilized in covalent linkages and which may, therefore, coordinate with water molecules by the donation of electrons or formation of a hydrogen bond as indicated on top of page 32.

There is also the possibility of coordination with water molecules by the acceptance of electrons from the oxygen atoms of water molecules onto hydrogen atoms linked to nitrogen or oxygen atoms. This seems a less probable mode of coordination with the exception of the ionised amino group, where the presence of a positive charge will favor the acceptance of electrons. The number of water molecules which will be held in this way by each group can not be assessed, but is not likely to be large. Allowing one water molecule to each possible point of coordination on the side chains and backbones of the gelatin molecule, a rough calculation shows that taking the molecular weight



of gelatin at 34,500, one molecule could coordinate with about 900 water molecules. One gram of dry gelatin holds 0.5 g. of closely bound water which gives a figure of 960 water molecules to one molecule of gelatin. These figures, though rough, encourage the belief that closely bound water is chemically bound to the protein. Coordination with water by the donation or acceptance of electrons is characteristic of uncharged groups, and will, therefore, occur at all values of hydrogen-ion concentration or salt concentration. Further evidence of the chemical nature of the binding may be deduced from the observation that the chemically bound water of egg albumin corresponds closely to the water of egg albumin crystals (Moran, 1935).

The problem of bound water is one of considerable importance, both in animal and plant physiology. It is not fixed throughout the life of the animal or plant. For instance, the amount of bound water is greatest in young animals and decreases with age. In plants it may also vary with the season.

It is hardly necessary to remind a meeting of cereal chemists of the work of Newton and Gortner (1922) on bound water and winter hardiness in wheats. The wheat *Minhardy* which has a high per-

centage of bound water in its winter leaves can resist the freezing temperatures of the cold season; the wheat Fulcaster, in which the leaf proteins have a lower percentage of bound water, can not do so. A high percentage of bound water enables a plant to resist desiccation not only by freezing, but also by drouth, as was shown by Newton and Martin (1930) in their work on drouth-resistant grasses.

Summary

Proteins imbibe water and hold it in two different forms which may be distinguished as loosely bound and firmly bound water.

The amount of total water bound varies with the activity of the water in the system.

The loosely bound water falls with decreasing activity of water, until the latter falls to about 0.7. At lower activities, 0.7 to 0.2, only the firmly bound water is present, which remains constant in amount over this range. Below an activity of 0.2, the firmly bound water is given up.

The loosely bound water can be removed by freezing, by direct pressure, or by evaporation.

The forces holding the loosely bound water are the interionic forces arising from the multipolar nature of the protein zwitterion.

It is suggested that the forces holding the closely bound water are chemical in nature, and are due to the formation of a coordinate link or hydrogen bond between the water molecules and the oxygen and nitrogen atoms of the protein. It is suggested that the presence of direct carbonyl-imino linkages between the polypeptide backbones of adjacent molecules, such as occur in fibers, reduces the amount of closely bound water.

The significance of these results in animal and plant physiology is indicated.

Literature Cited

- Astbury, W. T., and Lomax, R.
1935 An X-ray study of the hydration and denaturation of proteins. *J. Chem. Soc.* 846-851.
- , Dickinson, S., and Bailey, K.
1935 The X-ray interpretation of denaturation and the structure of the seed globulins. *Biochem. J.* 29: 2351-2360.
- Brooks, J.
1934 The vapour pressure isotherm of muscle. *Proc. Roy. Soc. B*, 114: 258-272.
- Cohn, E. J.
1936 Influence of the dielectric constant in biochemical systems. *Chem. Rev.* 19: 241-273.
- , McMeekin, T. L., Edsall, J. T., and Blanchard, M. H.
1933 The electrical forces in systems containing biological components. II. Molal volumes of amino acids, proteins, and certain related substances. *J. Biol. Chem.* 100: xxviii-xxxii.

- Greenberg, D. M., and Greenberg, M. M.
1933 Ultrafiltration. II. "Bound" water (hydration) of biological colloids. *J. Gen. Physiol.* 16: 559-569.
- Hampton, W. F., and Mennie, J. H.
1932 Heat capacity measurements on gelatin gels. *Can. J. Research* 7: 187-197.
- Hardy, W. B.
1905 Colloidal solution. The globulins. *J. Physiol.* 33: 251-337.
- Hatschek, Emil
1936 A direct demonstration of bound water in gelatin gel. *Trans. Faraday Soc* 32: 787-789.
- Jones, I. D., and Gortner, R. A.
1932 Free and bound water in elastic and non-elastic gels. *J. Phys. Chem.* 36: 387-436.
- Katz, J. R.
1917 Die Gesetze der Quellung. Eine biochemische und kolloid-chemische Studie. 1. Teil: Die Quellung in Wasser ohne Komplikationen. *Kolloidchem. Beih.* 9: 1-182.
- Lloyd, D. J., and Phillips, Henry.
1933 Protein structure and protein hydration. *Trans. Faraday Soc.* 29: 132-148.
- and Moran, T.
1934 Pressure and the water relations of proteins. I. Isoelectric gelatin gels. *Proc. Roy. Soc. A* 147: 382-395.
- Moran, T.
1926 The freezing of gelatin gel. *Proc. Roy. Soc. A* 112: 30-46.
1932 The hydration or combined water of gelatin. *Ibid.* A 135: 411-428.
1935 Bound water and phase equilibria in protein systems: Egg albumin and muscle. *Ibid.* B 118: 548-558.
- Newton, R., and Gortner, R. A.
1922 A method for estimating hydrophilic colloid content of expressed plant tissue fluids. *Botan. Gaz.* 74: 442-446.
- and Martin, W. M.
1930 Physico-chemical studies on the nature of drought resistance in crop plants. *Can. J. Research* 3: 336-427.
- Wyman, Jeffries, Jr.
1936 The dielectric constant of solutions of dipolar ions. *Chem. Rev.* 19: 213-239.

REPORT OF THE 1936-37 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

WALTER REIMAN, *Chairman*

Kroger Food Foundation, Cincinnati, Ohio

(Read at the Annual Meeting, May 1937)

The report of the 1935-36 Committee on Testing Biscuit and Cracker Flours (*Cereal Chem.* 13: 755-769) recommended that:

Work be repeated on other flours, subjecting the viscosity and baking tests to further examination employing essentially the technique therein outlined, and that a start be made to establish a definite correlation between laboratory and actual shop results by means of carefully controlled laboratory tests and practical tests on a commercial basis.

The present committee directed its activities toward carrying out the recommendations of the previous committee.

Plan of Procedure

Four flours representing respectively the maximum and minimum ranges for cracker dough and cracker sponge flours, as measured by the MacMichael viscosimeter (see Bohn, 1935), were selected for laboratory testing by members of the committee and also for the commercial shop tests.

The four flours were twice submitted to the collaborators for testing, but were coded so as not to reveal that identical flours were being tested in each series.

On the first series of tests the committee members were asked to determine the moisture, ash and protein contents of the flours, to measure their viscosity, and to classify the flours as to cracker dough, cracker sponge, or biscuit types on the basis of these results.

For the second series of tests the collaborators were asked to bake duplicate "pup" loaves from the flours and again to classify the flours on the basis of the baking test.

In order to check the classification of the flours on the basis of the baking test, loaves were baked from the four flours at the Kroger Food Foundation and sent to three members of the committee who were asked to score the loaves and classify the flours from which they were baked using the same methods of interpretation they applied to their own loaves.

For the cracker bakes on the commercial shop scale, each of the two sponge flours was combined with each of the two dough flours to

make four different types of cracker mixes. These cracker bakes were made three times each, with intervals of one month between the three series of bakes. A one-pound package of each type of cracker was sent to the collaborators for scoring after each bake. A chemical and physical analysis was also made on the four crackers of each bake at the Kroger Food Foundation.

Materials and Methods of Study

MATERIALS USED

The four flours used in this investigation and which shall be designated as Nos. 1, 2, 3, and 4 were of the following types.

No. 1. A strong soft winter wheat flour milled from southern Illinois wheat. It is generally used as a cracker sponge flour and represents the maximum in the viscosity range for this type of work.

No. 2. A moderately strong soft winter wheat flour milled from northern Indiana wheat. Normally finds its best application as a cracker sponge flour but is also satisfactory as a cracker dough or cookie flour. This flour is representative of the minimum in the viscosity range for cracker sponge flours.

No. 3. This is a weak soft winter wheat flour milled from central Ohio wheat. This flour, which is representative of the softest type of cracker dough flour, gives its best performance when used as such or as a cookie flour.

No. 4. This is a moderately strong soft winter wheat flour but not as strong as No. 2. This flour which was milled from southern Indiana wheat is representative of the strongest type of cracker dough flour and performs best when used for this purpose or as a cookie flour.

METHODS OF STUDY

Baking test: The baking test used was that of Werner, as described in Cereal Chemistry, volume 5, page 158, with variations as noted under Method B, Cereal Chemistry, volume 13, page 757.

The terminology used in describing the test loaves is also given under the last reference cited above.

Viscosity test: The viscosity test used was procedure No. 2, as suggested by Bayfield in Cereal Chemistry, volume 13, page 786.

Shortometer test: The Bailey Shortometer was used in this work. The shortometer readings recorded in the tables are in units of 1/32 pounds.

Commercial Scale Cracker Bakes

All of the crackers were baked on the basis of 5 barrels of flour to each mix. In each unit, 50% of the flour was of the cracker sponge type and 50% was of the cracker dough type.

Sixty percent or 3 barrels of the total amount of flour used went into the sponge along with 54% of the shortener, about 85% of the water, and all of the yeast. The 3 barrels of flour in the sponge were composed of 2½ barrels, or all of the sponge-type flour and 1/2 barrel or 20% of the dough-type flour. On the first two bakes, which are

designated respectively as A and B in this report, the sponges were fermented for 20 hours at 78° F. and 60 to 66% relative humidity. Due to the shop schedule at the time of the third bake, designated as bake C in this report, the sponge time was reduced to 17 hours. The temperature and relative humidity were the same as on bakes A and B.

To make up the dough, the fermented sponge was mixed with the two remaining barrels of the dough-type flour, 46% of the shortener, salt, malt, enough water to produce the proper dough consistency, and sodium bicarbonate. The quantity of sodium bicarbonate used was varied within narrow limits according to the temperature of the fermented sponge. The doughs were proofed for four hours and then baked into 2-inch square crackers at 540° F.

The crackers from each bake were scored by the collaborators, using the following method of evaluation.

CRACKER SCORING

Appearance 20 points

3 Symmetry of edge	3 Even, 2 uneven, 1 fish mouth, burnt
3 Shape	3 Even, 2 uneven, 1 buckled-cupped
5 Evenness of bake and color	5 Even, 3 uneven, 1 very uneven
3 Top color	3 Excellent, 2 fair, 1 poor
3 Bottom color	3 Good, 2 fair, 1 burnt, 0 dirty, floury
3 Bottom appearance	3 Good blisters, 2 fair, 1 too few, too many

Texture 50 points

10 Flakiness	10 Very flaky, 8 flaky, 6 compact, 3 very compact
15 Crispness	15 Crisp, 10 soft, 5 very soft
15 Tenderness	15 Very tender, 12 tender, 8 tough or flinty, 4 very tough
5 Chipping	5 Not chippy, 3 slightly chippy, 1 chippy
5 Color of crumb	5 White, 3 creamy, 1 grey

Flavor 30 points

20 Quality	20 Excellent, 15 good, 10 fair, 5 poor
5 Salt	5 Good, 3 too much, too little
5 Soda	5 Good, 3 too much, too little

In addition to scoring the crackers, the collaborators measured their pH by spotting them with either phenol red or brom thymol blue, determined the number of crackers per pound, and also the height of ten crackers.

An analysis for moisture, protein, and fat was made on each batch of crackers in addition to the shortometer test for tenderness. The shortometer figure recorded for each cracker represents the arithmetical average of 200 readings.

Experimental Results

Tables I, II, III, and IV record the analytical and viscosity results obtained by the collaborators on the four flours used in this investiga-

TABLE I
RESULTS OF COLLABORATIVE TESTS WITH FLOUR No. 1

Collaborator	% H ₂ O	% Ash as received	% Ash at 15% H ₂ O	% Protein as received	% Protein at 15% H ₂ O	Viscosity ° McM.		
						No acid	1 c.c.	7 c.c.
Pearl Brown	11.18	0.373	0.357	9.72	9.30			
C. C. Armuth	10.50	0.376	0.357	9.79	9.30	18	69	92
C. F. Evert	11.50	0.36	0.346	9.42	9.05	10	19 ¹	97
Jan Micka	9.90 ¹	0.38	0.359	9.90	9.34	12	54	96
C. O. Oppen		0.365		9.78		10	50	94
Kroger Foundation	12.46	0.380	0.369	9.55	9.28	14	58	89
Mean	11.41	0.372	0.358	9.70	9.25	13	56	94
Maximum	12.46	0.380	0.369	9.90	9.34	18	69	97
Minimum	9.90	0.360	0.346	9.42	9.05	10	19	89

¹ Not included in averages.

Comments

Pearl Brown: "Too strong even for cracker sponges."²

Messrs. Armuth and Knudson: "A cracker sponge flour."

C. F. Evert: "A cracker sponge flour."

Jan Micka: "A medium strong cracker sponge flour. The shorter extraction is responsible for the very high viscosity."

C. O. Oppen: "A cracker sponge flour."

Kroger Foundation: "A satisfactory cracker sponge flour."

² From average of viscosities.

TABLE II
RESULTS OF COLLABORATIVE TESTS WITH FLOUR No. 2

Collaborator	% H ₂ O	% Ash as received	% Ash at 15% H ₂ O	% Protein as received	% Protein at 15% H ₂ O	Viscosity ° McM.		
						No acid	1 c.c.	7 c.c.
Pearl Brown	11.09	0.345	0.330	8.36	8.00			
C. C. Armuth	10.60	0.346	0.329	8.37	7.96	18	48	62
C. F. Evert	11.10	0.334	0.320	8.15	7.79	10	45	60
Jan Micka	9.50 ¹	0.350	0.329	8.54	8.02	16	40	60
C. O. Oppen		0.490 ¹		8.40		11	38	63
Kroger Foundation	12.60	0.340	0.331	8.13	7.91	14	44	60
Mean	11.35	0.343	0.328	8.33	7.94	14	44	61
Maximum	12.60	0.490	0.320	8.54	8.02	18	48	63
Minimum	9.50	0.334	0.331	8.13	7.79	10	38	60

¹ Not included in averages

Comments

Pearl Brown: "A cracker sponge flour."²

Messrs. Armuth and Knudson: "Either a cracker sponge or cracker dough flour. Would probably work well in either case or as a topping flour for cracker dough sponge."

C. F. Evert: "A cracker sponge flour."

Jan Micka: "A cracker dough and cookie flour."

C. O. Oppen: "A cracker sponge or dough flour."

Kroger Foundation: "Best suited for use in cracker sponges but could also be used in cracker doughs."

² From average of viscosities.

TABLE III
RESULTS OF COLLABORATIVE TESTS WITH FLOUR NO. 3

Collaborator	% H ₂ O	% Ash as re- ceived	% Ash at 15% H ₂ O	% Pro- tein as received	% Pro- tein at 15% H ₂ O	Viscosity ° Mcm.		
						No acid	1 c.c.	7 c.c.
Pearl Brown	11.00	0.387	0.370	8.40	8.02			
C. C. Armuth	10.60	0.380	0.361	8.30	7.89	14	24	35
C. F. Evert	11.10	0.374	0.358	8.24	7.88	10	20	38
Jan Micka	9.80 ¹	0.396	0.373	8.50	8.01	12	20	40
C. O. Oppen		0.410		8.42		8	16	35
Kroger Foundation	12.05	0.390	0.377	8.22	7.95	11	19	35
Mean	11.19	0.390	0.368	8.35	7.95	11	20	37
Maximum	12.05	0.410	0.377	8.50	8.02	14	24	40
Minimum	9.80	0.374	0.358	8.22	7.88	8	16	35

¹ Not included in averages.

Comments

Pearl Brown: "A cookie flour. This might border upon dough flour performance."²

Messrs. Armuth and Knudson: "A cracker dough flour."

C. F. Evert: "A cracker dough or cookie flour."

Jan Micka: "A cracker dough and cookie flour."

C. O. Oppen: "A cracker dough and cookie flour."

Kroger Foundation: "Can be used as a cracker dough flour and also for hard sweet goods."

² From average of viscosities.

TABLE IV
RESULTS OF COLLABORATIVE TESTS WITH FLOUR NO. 4

Collaborator	% H ₂ O	% Ash as re- ceived	% Ash at 15% H ₂ O	% Pro- tein as received	% Pro- tein at 15% H ₂ O	Viscosity ° Mcm.		
						No acid	1 c.c.	7 c.c.
Pearl Brown	11.39	0.397	0.381	8.44	8.10			
C. C. Armuth	10.60	0.384	0.365	8.42	8.01	14	35	60
C. F. Evert	11.70	0.373	0.359	8.20	7.89	10	24	58
Jan Micka	9.70 ¹	0.402	0.378	8.64	8.14	10	25	60
C. O. Oppen		0.410		8.38		8	20	60
Kroger Foundation	12.78	0.380	0.370	8.16	7.96	11	28	55
Mean	11.62	0.391	0.371	8.37	8.02	11	26	59
Maximum	12.78	0.410	0.381	8.44	8.14	14	35	60
Minimum	9.70	0.373	0.359	8.16	7.89	8	20	55

¹ Not included in averages.

Comments

Pearl Brown: "A cracker dough flour. This might be a borderline flour in the direction of a cracker sponge type."²

Messrs. Armuth and Knudson: "A borderline cracker sponge flour. Would probably work better as a cracker dough flour or as a topping flour for cracker dough sponge."

C. F. Evert: "A cracker dough flour."

Jan Micka: "A stronger type cracker dough flour."

C. O. Oppen: "A cracker dough flour."

Kroger Foundation: "This flour is on the border between a cracker sponge and cracker dough type, but would probably be more satisfactory when used for cracker doughs."

² From average of viscosities.

tion. The classifications assigned to the flours, on the basis of the viscosity test, are also given in these tables. Tables V and VI report

TABLE V
BREAD SCORES ON FLOURS 1 AND 2

Characteristic	Flour 1				Flour 2			
	Pearl Brown	C. F. Evert	C. O. Oppen	Kroger Foundation	Pearl Brown	C. F. Evert	C. O. Oppen	Kroger Foundation
Oven spring	Medium large	Small	Medium	Large	Large	Small	Medium	Large
Break and shred	Ragged	Slightly ragged	Fairly smooth	Slightly shell top	Very ragged	Slightly ragged	Torn	Fairly smooth
Crust color	Dark	Pale	Pale	Medium dark	Pale	Pale	Pale	Medium dark
Grain	Close, elongated	Close and elongated	Close, elongated	Close, elongated	Close, round	Coarse and elongated	Close, round	Close, elongated
Color of crumb	White gray	White	White	White	Cream gray	Slightly dull	Creamy white	White
Volume, c.c.		460	455	415		445	480	415
% Absorption	57	50	56	57	55	54	55	57

Comments

Pearl Brown—"A strong sponge type flour."

C. F. Evert—"Suitable for cracker sponging."

C. O. Oppen—"This is definitely a cracker sponge flour."

Kroger Foundation—"The strongest of the four flours. Satisfactory for use in cracker sponges."

"A cracker dough flour but strong enough for use in cracker sponges."

"Loaves baked from flours 2, 3, and 4 are so similar we can make no distinction between the flours."

"Satisfactory for use in both cracker sponges and cracker doughs."

"Not as strong as 1 but strong enough for use as a cracker sponge flour."

TABLE VI
BREAD SCORES ON FLOURS 3 AND 4

Characteristic	Flour 3				Flour 4			
	Pearl Brown	C. F. Evert	C. O. Oppen	Kroger Foundation	Pearl Brown	C. F. Evert	C. O. Oppen	Kroger Foundation
Oven spring	Medium large	Small	Medium	Large	Large	Small	Small	Large
Break and shred	Very ragged	Slightly ragged	Torn	Torn	Ragged	Slightly ragged	Slight	Fairly smooth
Crust color	Medium	Pale	Pale	Medium	Dark	Pale	Pale	Medium
Grain	Coarse	Slightly coarse	Close, round	Close, elongated	Very coarse	Coarse	Coarse	Close, elongated
Color of crumb	Cream	Dull	White	Creamy white	Dark cream	Dull	Dull creamy white	Creamy
Volume, c.c.		450	480	440		452	450	405
% Absorption	55	55	55	57	55	54	56	57

Comments

Pearl Brown—"A cookie flour."

C. F. Evert—See Table V.

C. O. Oppen—"Could be successfully used in both cracker sponges and doughs."

Kroger Foundation—"Stronger than 4, but softer than either 1 or 2. Most applicable for use as a cracker doughing flour."

"A cookie flour."

See Table V.

"A cracker doughing flour."

"The softest of the four flours. Suitable for use in cracker doughs and hard sweet goods."

the collaborators' scores on their own test loaves, while Tables VII and VIII record their scores on the loaves baked from the same flours

TABLE VII
COLLABORATORS' SCORES ON SAME LOAVES

Characteristic	Flour 1			Flour 2		
	Pearl Brown	C. F. Evert	C. O. Oppen	Pearl Brown	C. F. Evert	C. O. Oppen
Oven spring	Large	Small	Medium	Medium large	Small	Medium
Break and shred	Ragged	Ragged	Slightly torn	Ragged	Slightly ragged	Torn
Crust color	Medium	Medium	Medium	Slightly dark	Medium	Medium
Grain	Elongated	Close and elongated	Elongated	Close and elongated	Close and elongated	Small round
Color of crumb	White	White	Creamy white	Creamy white	Slightly dull	White
Volume c.c.		360	370		370	395
% Absorption		57			57	

Comments

Pearl Brown—"A cracker dough flour. Could also be used in the sponge."

"A cracker sponge flour."

C. O. Oppen—"A cracker sponge flour."

"A cracker dough flour."

TABLE VIII
COLLABORATORS' SCORES ON SAME LOAVES

Characteristic	Flour 3			Flour 4		
	Pearl Brown	C. F. Evert	C. O. Oppen	Pearl Brown	C. F. Evert	C. O. Oppen
Oven spring	Large	Small	Medium	Medium, large	Small	Medium
Break and shred	Very ragged	Shell	Torn	Very ragged	Smooth	Torn
Crust color	Pale	Pale	Pale	Dark	Medium	Brown
Grain	Coarse	Slightly coarse	Coarse	Elongated	Close and round	Small round
Color of crumb	Creamy gray	Dull	Dull white	Gray white	Dull	Creamy dull
Volume c.c.		355	380		370	375
% Absorption		57			57	

Comments

Pearl Brown—"A cookie flour."

"A cookie flour."

C. O. Oppen—"A cracker dough or sweet goods flour."

"A cookie flour."

at the Kroger Food Foundation. These tables also include the collaborators' classifications of the flours on the basis of the baking test. Figures 1 and 2 are the photographs of the test loaves baked by the collaborators from the four flours used in this work.

The chemical and physical measurements of the crackers from each of the three bakes and of the flours as used at each bake are reported in Table IX. Table X summarizes the ranking of the crackers of each bake by means of the collaborators' scores and the shortometer readings.

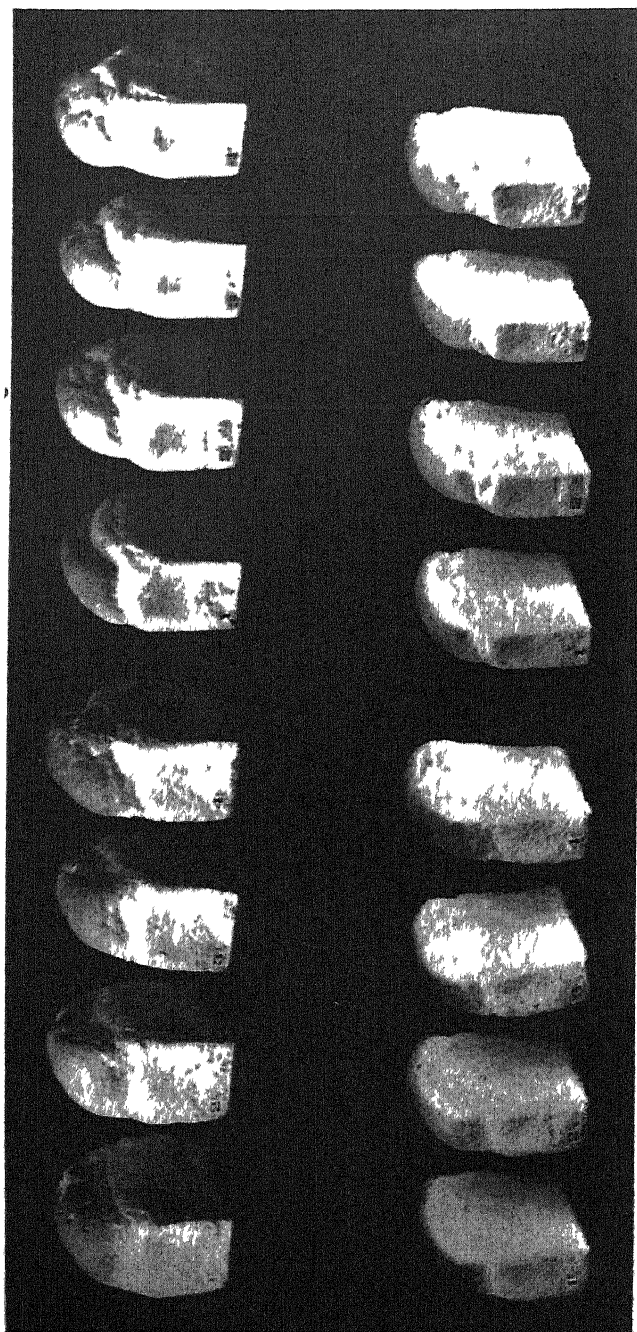


Figure 1 Loaves baked respectively by C O Oppen and at Kroger Food Foundation

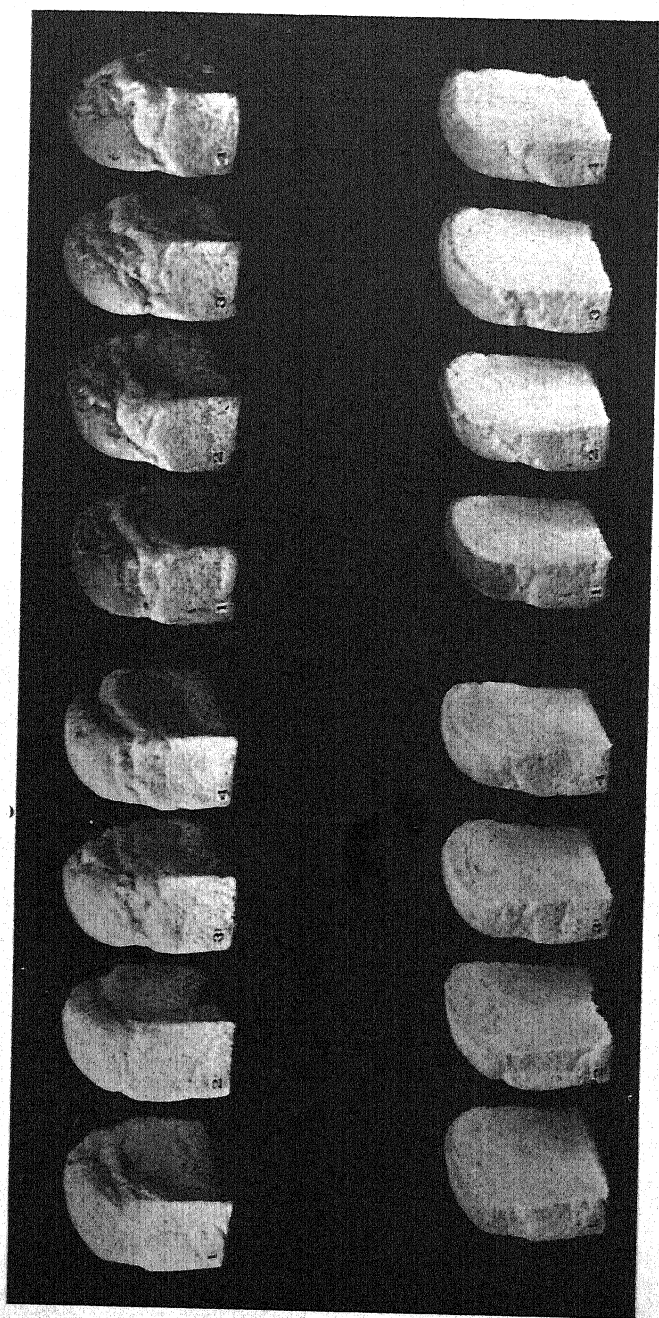


Figure 2. Loaves baked respectively by C. F. Evert and Pearl Brown.

TABLE IX
CHEMICAL AND PHYSICAL MEASUREMENTS ON CRACKERS AND ON FLOUR
AT EACH BAKE

Crackers								Flour							
Bake	Cracker	% Moisture	% Protein	% Fat	Shortometer reading	pH	Thickness 10 (in.)	No. lb.	No.	% Moisture	% Ash	% Protein	Viscosity		
													No acid	1 c.c.	7 c.c.
A	1	4.16	8.54	13.48	79.5	7.4	2 $\frac{1}{2}$ ₁₆	108	1	11.68	0.40	9.89	14	62	96
	2	4.60	8.23	13.22	88.8	8.2	3 $\frac{1}{8}$ ₁₆	110	2	11.84	0.40	8.11	14	46	60
	3	3.36	7.88	13.55	76.6	7.6	2 $\frac{3}{4}$ ₁₆	114	3	11.74	0.39	8.27	11	24	36
	4	3.38	7.95	13.37	84.5	7.8	2 $\frac{1}{2}$ ₁₆	114	4	12.61	0.35	7.80	12	30	54
B	1	4.41	8.42	12.83	89.4	7.4	2 $\frac{1}{2}$ ₁₆	108	1	12.55	0.38	9.40	14	62	95
	2	5.63	8.23	12.66	96.6	7.4	3	109	2	12.73	0.34	7.94	15	44	55
	3	3.70	7.75	13.04	74.8	7.4	2 $\frac{3}{4}$ ₁₆	112	3	12.53	0.39	8.10	9	23	36
	4	4.02	7.60	13.12	77.7	7.4	2 $\frac{3}{4}$ ₁₆	113	4	12.94	0.39	8.12	11	24	53
C	1	5.12	8.50	12.54	91.4	8.4	3	108	1	13.16	0.38	9.47	14	59	98
	2	4.31	8.42	12.85	85.4	7.6	3 $\frac{1}{8}$ ₁₆	111	2	13.75	0.35	7.78	15	44	57
	3	4.34	7.80	13.06	81.1	8.2	2 $\frac{7}{8}$ ₁₆	111	3	12.82	0.41	8.07	11	21	35
	4	4.28	7.68	12.84	82.9	8.2	3 $\frac{1}{8}$ ₁₆	109	4	13.65	0.41	7.98	10	33	57
Fat on flour															
									No.	1	2	3	4		
									% Fat 15% H ₂ O	0.48	0.91	1.10	0.80		

¹ Unit of measurement 1/32 pound.

TABLE X
RANKING OF CRACKERS FROM COLLABORATORS' SCORES AND SHORTOMETER TESTS

	Bake A				Bake B				Bake C			
Ranking on Cracker No.	1	2	3	4	1	2	3	4	1	2	3	4
Pearl Brown	1	4	2	3	2	3	1	3	3	2	1	4
C. C. Armuth	2	4	2	1	2	1	2	2	3	1	2	4
C. F. Evert	2	4	3	1	4	1	2	3	2	2	4	1
Jan Micka					2	4	1	3	3	4	1	1
C. O. Oppen					3	4	1	3				
W. S. Culver	1	1	2	1	3	4	1	2	4	2	1	2
W. Reiman	3	4	1	2	2	4	1	3	3	4	1	2
Total	9	17	10	8	18	21	9	19	22	15	10	14
Rank	2	4	3	1	2	4	1	3	4	3	1	2

	Bake A				Bake B				Bake C			
Ranking on Cracker	1	2	3	4	1	2	3	4	1	2	3	4
Rank	2	4	1	3	3	4	1	2	4	3	1	2

Composite of Score and Shortometer Readings

Test	Collaborator's ranking				Shortometer ranking			
Cracker No.	1	2	3	4	1	2	3	4
Total	8	11	5	6	9	11	3	7
Rank	3	4	1	2	3	4	1	2

Discussion of Results

The analytical results reported by the collaborators on the flours used in this investigation are in good agreement with the exception of the figures on moisture content. The discrepancies in the moisture results on the flours do not reflect weaknesses in the analytical procedure but rather the condition encountered in transit and in the laboratories while awaiting analysis.

The ash results indicate that flour No. 2 is lowest on ash, flours Nos. 3 and 4 are about the same and highest on ash, with flour No. 1 in between these two extremes but closer to the higher figure. The figures on protein content show flour No. 1 to be about 1.3% higher on protein than flours Nos. 2, 3, and 4, which are practically the same.

With the exception of flour No. 1, the viscosity results reported for the flours are in fairly good agreement, with the magnitude of the difference between collaborators' readings on the same flour about like that reported in this work last year (*loc. cit.*). The only reason that the viscosity figures on flour No. 1 show a greater difference than on the other flours is because of the low reading reported by one collaborator. That this reading is an exception is indicated by the viscosity results on flour No. 1 reported by this same collaborator in Table IX. These figures compare favorably with the other results and if used would reduce the magnitude of the difference in viscosity readings to within that reported on flours Nos. 2, 3, and 4. In using their viscosity results as a means of classifying the flours, the collaborators were able to arrive at almost unanimous opinions on the best use for each flour.

The results of scoring the test loaves, reported in Tables V and VI, show satisfactory agreement (except for the figures on volume) among the various collaborators. The differences in the figures on volume may be explained in part by the fact that all the collaborators did not use the same size of baking pan. Individual differences in molding and panning technique may also contribute to this condition. Despite the differences in appearance of the loaves as shown in Figures 1 and 2, the collaborators were able to reach practically the same conclusions regarding the best commercial application for each flour. However, two of the collaborators who participated in both the viscosity and baking tests reported that flour No. 4 was stronger than flour No. 3, on the basis of the viscosity test, but on the basis of their baking results, said that flour No. 3 was stronger than No. 4. This condition is analogous to that reported by this committee last year (*loc. cit.*) where two of the flours were ranked a degree higher in strength by means of the baking test than by the viscosity test.

Tables VII and VIII indicate that the collaborators were able to reach the same conclusions with respect to the flours from loaves baked by someone else as from their own loaves.

Considering the number of variables in commercial cracker baking, the analyses of the four crackers at the times of their respective bakes, as reported in Table IX, show a fair degree of consistency. This is exhibited by the trends in the results rather than in the absolute values themselves. Crackers Nos. 1 and 2 have, in the instance of each bake, a higher percentage of moisture and protein and a lower percentage of fat than crackers Nos. 3 and 4. The shortometer readings show higher results on each bake for crackers Nos. 1 and 2 than for crackers Nos. 3 and 4. The results also show that crackers Nos. 1 and 2 have greater spring as evidenced by their thickness and run fewer to the pound than crackers Nos. 3 and 4. On the second bake only were the pH readings of the four crackers the same. The analysis of the flours at the time of the different bakes indicates that they gained moisture while in storage but remained practically constant on viscosity. The percentages of ash and protein decrease with the increase in moisture.

Although the crackers from each bake were scored in detail by the collaborators, these results are not presented since they would only tend to confuse rather than clarify the work. The reason for this is the rather marked differences of opinion expressed by the collaborators in their numerical evaluations of cracker characteristics. Table X however, records the relative rankings of the crackers based on the collaborators' scores in comparison with the relative rankings on the basis of the shortometer test. The cracker which scored highest and gave the lowest shortometer reading was ranked first on the respective bases. When compared with one another, on the basis of collaborators' scores, the rankings of the crackers at the time of each bake do not show a very great degree of consistency. More comparable rankings are reported on the basis of the shortometer tests. On only one bake, the third, do the cracker rankings, on the basis of collaborators' scores and shortometer tests, exactly agree. However, when the rankings at the time of each bake are composited on their respective bases the ranking of the crackers by means of the collaborators' scores and the shortometer tests is exactly the same. These show that cracker No. 3 is first, No. 4 second, No. 1 third, and No. 2 fourth.

On the basis of the viscosity test, cracker No. 3, which ranked first, is composed of the softer sponge and the softer dough flour; cracker No. 4, which ranked second, is made from the softer sponge and the stronger dough flour; cracker No. 1, which ranked third, is com-

posed of the stronger sponge and the softer dough flour; and cracker No. 2, which ranked fourth, is composed of the stronger sponge and the stronger dough flour.

Using the baking test as the criterion of strength, cracker No. 3 is composed of the softer sponge and stronger dough flour; cracker No. 4 is made from the softer sponge and the softer dough flour; cracker No. 1 is composed of both the stronger sponge and dough flours; and cracker No. 2 is made from the stronger sponge and softer dough flours. On the basis of either the baking or viscosity tests, as the measurement of flour strength, the quality of the crackers seems to be more dependent on the type of sponge flour than on the type of dough flour used in its formula.

A comparison of Tables IX and X shows that the best crackers have the least spring and lowest moisture content. This relationship is dependent on the types of flour used, since the best crackers were made from the softer flours which could be more easily baked out. The pH of a cracker is a determining factor in its spring, but the comparison still holds in bake B where all four crackers had the same pH.

Conclusions

As a result of its work this year, the Committee on Testing Biscuit and Cracker Flours concludes that:

While the relationship between cracker quality and types of flour as pointed out in this report is not definite nor are the data presented extensive enough to form precise conclusions, we believe there is sufficient evidence to indicate that the methods used in evaluating cracker flours give useful information and should be continued.

Further confirmation is given to the work of previous committees in their efforts to determine the relative strengths of biscuit and cracker flours by means of the baking and/or viscosity tests.

Recommendations

The committee therefore recommends that:

More work of the type herein reported be done to build up a greater volume of data to correlate more accurately the laboratory analyses of biscuit and cracker flours with their shop performance.

Experimental work be undertaken to explain and, if possible, correct the apparent discrepancy between the baking and viscosity tests for determining the relative strengths of biscuit and cracker flours.

Acknowledgments

This report is the result of the cooperative efforts of a number of individuals and appreciation for their work is hereby expressed. The

members of the committee who participated in the collaborative tests were: Miss Pearl Brown, C. C. Armuth, C. F. Evert, Jan Micka, C. O. Oppen, and the writer. Thanks are also due R. M. Knudson of Ogden, Utah for his work on the viscosity test.

The writer is indebted to George Garnatz for his suggestions in planning this work and much help in writing the report and to W. S. Culver for conducting the shop tests and making many of the analyses. Appreciation for preparing this report is due Mrs. Dorothy Shriver. The work of the Cereal Laboratory of the Kroger Food Foundation on the baking tests is acknowledged.

The flours used in this investigation were obtained through the efforts of H. H. Wurtz, Purchasing Agent for the Kroger Grocery and Baking Company. The shop tests were conducted at Kroger's Columbus Cracker Bakery through the courtesy of R. F. Lovell.

Literature Cited

Bayfield, E. G.

1936 Report of the subcommittee on viscosity. *Cereal Chem.* 13: 773-788.

Blish, M. J.

1928 Standard experimental baking test. *Cereal Chem.* 5: 158-161.

Bohn, R. M.

1934 Report of the committee on testing biscuit and cracker flours. *Cereal Chem.* 11: 661-668.

1935 Report of the committee on testing biscuit and cracker flours. *Ibid.* 12: 544-551.

Reiman, Walter

1936 Report of the 1935-36 committee on testing biscuit and cracker flours. *Cereal Chem.* 13: 755-769.

THE QUANTITATIVE DETERMINATION OF BROMINE IN BROMATED FLOURS¹

W. F. GEDDES and F. H. LEHBERG

Board of Grain Commissioners, Grain Research Laboratory, Winnipeg, Canada

Read at the Annual Meeting, May 1937

Recently this laboratory had occasion to investigate an extended series of competing flours obtained from several countries where the baking methods in use call for small percentages of yeast. When baked by the A. A. C. C. basic procedure many of the samples yielded loaves possessing decided overfermented characteristics, indicating that the flours had been heavily treated to increase the rate of gluten development and thereby render them more suitable for the baking procedures for which they were intended. Qualitative tests showed that these samples had been bromated and it became of importance to determine the quantities present.

As far as the quantitative determination of bromine in flour or flour improvers is concerned, only two methods, that of Meyer (1931) and of Kulman (1934, 1934a) were found in the literature. The method of Meyer (1931) which is described in the A. A. C. C. Book of Methods (1935) is a macro-volumetric method intended for the determination of bromates in commercial flour improvers; on the other hand, Kulman's method is described for treated flours containing 0.005% KBrO_3 and depends upon its separation, along with other added chemicals such as persulphates and iodates, by a flotation procedure involving centrifuging a suspension of the sample in chloroform or carbon tetrachloride. The quantity of potassium bromate isolated is determined by treatment with KI and HCl, overtitration of the liberated iodine with 0.01N $\text{Na}_2\text{S}_2\text{O}_3$ and the excess back-titrated with 0.01N KI. The presence of KBrO_3 must be identified before the method can be applied, and in flours containing persulphates or iodates these constituents must be determined separately in aliquots of the final solution.

During the last few years considerable effort has been devoted to the development of reliable micro methods for the estimation of bromine in biological material but it is not proposed to give here a complete survey of the extensive literature.

¹ Published as paper No. 123 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.

All the procedures involve an initial destruction of the organic matter by wet or dry ashing followed by selective oxidation of the inorganic halides, the quantitative separation of bromine and its determination by titrimetric or colorimetric methods. A study of the literature reveals that the problem of bromine estimation in such materials is by no means simple and recent research has shown that a number of factors including loss of bromide during ashing, its incomplete oxidation, partial oxidation of the chlorides present and incomplete separation of the bromine, rendered many of the earlier methods unreliable. While the separation of bromides and iodides may be readily accomplished with a variety of oxidizing agents, the former being converted to bromine and the latter to iodate, the separation from chlorides is much more difficult and it is only recently that selective oxidation of chlorides and bromides has been satisfactorily accomplished.

Bobtelsky and Rosowskaya-Rossienskaya (1931) and Evans (1931) found that bromide may be rapidly and completely oxidized to bromine without oxidation of chlorides in a chromic-sulphuric acid mixture of definite concentration, and Yates' (1933) micro method for the determination of bromine in blood is based on this principle. Protein is first removed by precipitation with tungstic acid, the filtrate treated with potassium hydroxide, evaporated to dryness and incinerated at 500° C. for 20 minutes in a nickel crucible. The mixed halides are then oxidized with a chromic-sulphuric acid mixture of such concentration that the bromide is completely oxidized to bromine in the cold, while the chloride remains unaffected and any iodide present is converted to iodate. The bromine is removed by aeration, absorbed in starch-iodide solution and the liberated iodine titrated with 0.001N $\text{Na}_2\text{S}_2\text{O}_3$ from a microburette. Yates states that the method is accurate to within $2 \gamma^2$ for amounts ranging from 5 to 1,000 γ of bromine.

For the micro determination of bromine in blood, Francis and Harvey (1933) destroy the organic matter by ignition with KOH under controlled conditions and employ a chromic-phosphoric acid mixture for liberation of bromine, which is recovered by aeration in all-glass apparatus and determined in the usual manner by absorption in starch iodide solution and titration of the liberated iodine with 0.002N $\text{Na}_2\text{S}_2\text{O}_3$. These workers report a recovery of bromine within $\pm 10\%$ of the theoretical and emphasize the necessity of pure reagents, scrupulous cleanliness and attention to details. High results may be obtained with materials containing iodine.

²One gamma equals one one-thousandth of a milligram.

Leipert and Watzlawek (1934) destroy organic matter by a wet ashing procedure using a chromic-sulphuric acid mixture in a closed apparatus, the liberated bromine and chlorine being led into NaOH solution where the bromide and hypobromite are oxidized by hypochlorite to bromate which is determined iodometrically.

Several colorimetric methods are also described in the literature. Scott (1925) states that small amounts of bromine may be determined colorimetrically by means of magenta reagent which produces a violet to reddish-violet coloration in the presence of bromine. Tod (1933) developed a colorimetric method for the estimation of the bromide content of body fluids based on the production of a reddish-brown solution of auric bromide when auric chloride and bromide solutions are mixed. More recently Stenger and Kolthoff (1935) have described a colorimetric method based on the fact that phenol red, with a colour change from yellow to red over the pH interval 6.4 to 8.0, reacts with very dilute hypobromite in weakly alkaline solution to form an indicator of the brom-phenol blue type which changes from yellow to blue-violet over the pH range 3.2 to 4.6. Concentrations of 0 to 4000 γ of bromine, present as bromide, can be estimated with an accuracy of 15 to 20%; the method is not applicable to solutions containing over 18,000 γ , since in concentrations from 18,000 to 30,000 γ nearly constant color is produced, while above 30,000 the indicator is attacked and fades.

Experimental

Since the quantity of potassium bromate present in treated wheat flours rarely exceeds 0.005% which corresponds to 0.00239% bromine, microchemical methods must be employed for its estimation. Meyer's (1931) macro-volumetric method for the determination of bromates in flour improvers is obviously unsuited for their estimation in treated flours.

Experiments with Kulman's (1934, 1934a) flotation method for separating the added bromate from the flour showed it to be quite unreliable, particularly for concentrations below 0.003%, the results being uniformly low with unsatisfactory replicability. The colorimetric methods of Scott (1925) employing magenta reagent and of Tod (1933), which is based on the production of auric bromide, were next investigated and abandoned. With the magenta test, difficulty was experienced with adsorption of the violet colour of the chloroform layer at the chloroform-water interface. The Tod method gave low and variable results. As Stenger and Kolthoff (1935) claim only a relatively low accuracy for their method, it was not studied.

The methods available, therefore, resolved themselves into those involving the micro-determination of bromine which require the destruction of the organic material and the quantitative separation of bromine from chlorine and iodine. A survey of the literature indicated that the latter could be satisfactorily accomplished by the partial oxidation procedure outlined by Yates (1933). Preliminary experiments showed this to be the case and the method finally adopted for this and subsequent stages of the determination differed from that of Yates in only a few details.

The reaction apparatus employed by Yates consisted of a 100 c.c. conical flask fitted with a two-holed rubber stopper, the air inlet tube being carried down to the bottom of the flask to ensure maximum aeration. The reaction flask is connected to an absorber consisting of a test tube (10 X 1 cm.) fitted with a two-holed stopper carrying an exit and an inlet tube drawn out to a fine capillary, which extends to within 2 mm. from the bottom of the test tube; only one absorber is used but after aeration for two hours the tube is renewed and the aeration continued for a further three hours. Using a similar set-up and working with potassium bromide solutions of known concentration, it was found that from 4 to 5% greater recovery could be obtained by the addition of a second absorption tube. With this modification, the recovery of bromine present in amounts corresponding to those in treated flours was in the order of 95 to 96%, which is very satisfactory for work of this kind.

However, Francis and Harvey (1933) emphasized the importance of keeping the apparatus free from minute traces of organic matter and specifically warned against the use of rubber joints. They employed an all-glass apparatus comprising a reaction vessel and two small absorption tubes, the ground glass joints being lubricated with distilled water only. In view of their experience, it appeared that higher recoveries and better replicability might be possible with an all-glass apparatus so designed as to eliminate as many connections as possible and to permit of the titrations being carried out directly in the apparatus. Accordingly, at the request of the authors, a special Pyrex glass reaction and absorption vessel with standard taper ground joints was designed and constructed by Binnington (1937)³ for this purpose, the constructional details of which are shown in Figure 1. In the analysis of potassium bromide solutions the use of this apparatus gave recoveries between 98 and 100% with relatively low variability between replicates.

These results on potassium bromide solutions showed that the modified Yates oxidation and recovery procedures were eminently

³ D. S. Binnington.

satisfactory and attention was next directed to the determination of potassium bromate in wheat flours, which first involves its separation from the large bulk of organic matter. For the destruction of organic matter in the micro-determination of bromine in blood, Francis and

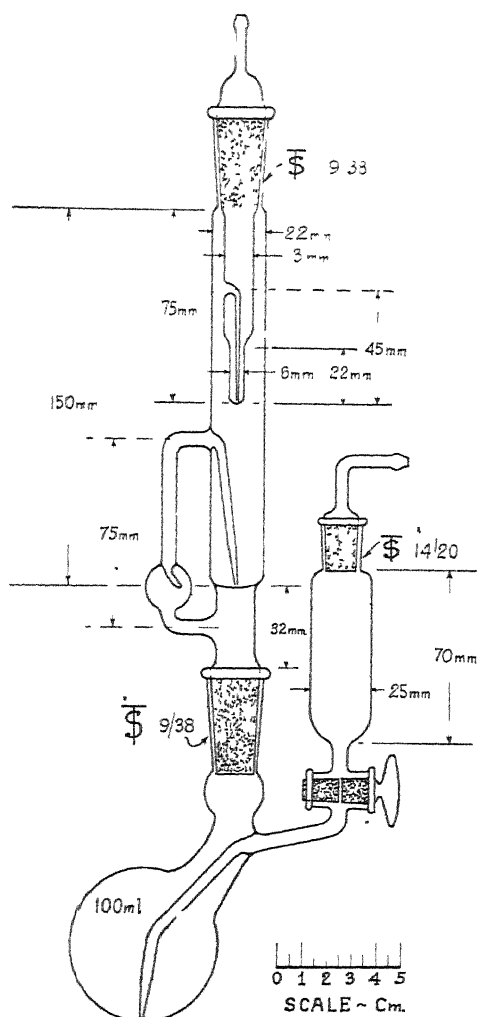


Figure 1. Constructional details of Pyrex glass reaction vessel.

Harvey (1933) add a definite quantity of potassium hydroxide solution and incinerate first in a nickel crucible and finally in a platinum one at 480° to 500° C. After the preliminary ignition the carbonized mass is digested with water on a steam bath, the carbon separated by

filtration, which is again incinerated, digested and filtered. The two filtrates are combined, evaporated and incinerated in platinum.

A series of bromated flours containing 1, 2, 3 and 5 mg. of KBrO_3 per 100 g. of flour was prepared and the bromine content determined on a 20-g. sample by the modified Yates method after ashing by a procedure similar to that outlined by Francis and Harvey (1933). The percentage recovery varied from 78 to 92%, the highest value being obtained with the samples containing 0.005% KBrO_3 . Several modifications were tried without material improvement. The complete destruction of such large quantities of organic matter by this process is difficult and tedious and, in view of the low results, it was decided to try leaching the soluble bromate from the main flour mass before ashing. This resulted in a satisfactory recovery, and the complete method as finally developed follows.

Method in Detail

Apparatus necessary

300 c.c. wide-mouthed, glass-stoppered bottles.

Mechanical shaker.

Centrifuge fitted with head for 100 ml. tubes and capable of being operated at 2,800 r.p.m.

100 c.c. glass centrifuge tubes.

Evaporating dish, capacity 300 c.c., preferably platinum.

Nickel or preferably platinum crucible with lip, capacity 75 c.c.

Mohr pipettes, graduated in 0.10 ml.

Binnington all-glass micro-reaction and absorbing vessel (Figure 1).

Micro-burette, capacity 5 ml.

Reagents

Potassium hydroxide solution, 10%.

Potassium nitrate solution, 0.1%.

Sulphuric acid—concentrated (C.P.).

Chromic-sulphuric acid mixture. Dissolve 20 g. chromic oxide (C.P.) in 120 c.c. distilled water and slowly add, with cooling, 40 c.c. concentrated sulphuric acid (C.P.).

Potassium iodide solution, 10%; freshly prepared.

Starch solution, 0.5%.

Sodium thiosulphate solution, 0.001N; prepared by dilution of 0.1N thiosulphate solution, which has been adequately aged.

Determination

Extraction: Weigh a 20-g. sample into a 300 c.c. wide-mouthed, glass-stoppered bottle, add 75 c.c. of freshly distilled water and stir thoroughly with glass rod to assist complete suspension, setting aside rod for further use. Add a few glass beads and shake for one hour in mechanical shaker. Carefully transfer suspension to a 100 c.c. centrifuge tube; centrifuge for approximately 15 minutes at 2,800 r.p.m. Filter supernatant solution through a filter paper, Watman No. 4, 11 cm., and collect filtrate in a 300 c.c. evaporating dish, preferably of platinum.

By means of the glass rod previously used, break up remaining dough mass in centrifuge tube with 40 c.c. of distilled water; transfer to the wide-mouthed bottle used in the first operation, by means of approximately 35 c.c. distilled water. Great care should be taken that a proper dispersion of the mass is obtained.

Shake for one hour and centrifuge as previously described, filter through the same paper used for the first filtration, wash with approximately 20 c.c. boiling distilled water, combining washings and filtrate in the 300 c.c. evaporating dish.

Add 2.5 c.c. of 10% KOH solution and evaporate on a steam bath to a volume of approximately 30 c.c. Transfer to a lipped nickel or platinum crucible (75 c.c. capacity), rinse evaporating dish thoroughly with boiling distilled water (the washings not to exceed 25 c.c. are collected in the crucible) and evaporate contents of crucible to dryness.

Ashing: Incinerate the crucible and its contents at 500° C. for 30 minutes and cool; break up the carbonized mass by means of a glass rod which is rinsed with approximately 10 c.c. of distilled water, collecting the rinsings in the crucible. Evaporate to dryness and again incinerate at 500° C. for 1½ hours.

If all the carbonaceous matter has not been destroyed, repeat the above procedure and if after the third incineration carbon is still present, moisten with 2 c.c. of a 0.1% KNO₃ solution, evaporate to dryness and ignite at 500° C. until completely ashed.

Oxidation, aeration and titration: The residue is dissolved and transferred to the reaction flask by means of 7 c.c. of distilled water ($\pm$ 0.1 c.c.) using a 3 c.c. and two 2 c.c. portions for this process. Place 3 c.c. of 10% KI solution and 4 drops of starch solution in the main absorption tube, 1 c.c. of KI solution and 2 drops of starch solution in the secondary trap and connect to the reaction flask.

Partially immerse reaction flask in an ice-water bath and by means of the side-tap funnel carefully and slowly add 2.5 c.c. of concentrated H₂SO₄, taking at least 10 minutes for this addition. To the cold solution in the reaction flask add rapidly by means of the tap funnel 4 c.c. of the chromic acid-sulphuric acid mixture. Air, aspirated through 25% KOH solution, is drawn through the reaction flask by means of a water pump at a rate of approximately one bubble per second. After three hours' aeration, during which period the apparatus is held at room temperature, disconnect the absorption unit, loosen the upper stopper, blow the contents of the trap into the main vessel and wash with two successive 1 c.c. portions of the 10% KI solution. Titration is carried out in the main absorber with 0.001N sodium thiosulphate solution. Wash and dry the absorption unit, recharge the absorbers, and continue the aeration for a further 2 to 2½ hours, after which period titrate the liberated iodine as before.

1 c.c. 0.001N thiosulphate = 0.0000799 gram bromine or 0.0001670 gram KBrO_3 . To calculate the percentage of KBrO_3 , multiply the thiosulphate titration in c.c. by 0.000835.

Discussion

It is essential that the evaporated extract be entirely free from moisture before ashing; otherwise trouble is experienced from decrepitation.

The ash should be finely divided before lixiviation and must be perfectly free from carbon. Traces of organic matter seriously influence the results.

In carrying out the oxidation, both direct sunlight and darkness must be avoided.

Scrupulous cleanliness and attention to detail are essential to secure reliable results. The volume of the titer must not be increased by washing down the sides of the absorbing vessel during titration; otherwise low results are secured.

Blank tests should be periodically conducted.

A single determination requires approximately eight hours and one analyst can conduct up to four determinations simultaneously.

Accuracy of the Method

The prepared flours containing 1, 2, 3 and 5 mg. of KBrO_3 per 100 of flour were analyzed in duplicate by the above procedure and the mean values obtained were 93.6, 94.8, 92.6 and 97.2%, respectively, of the theoretical. While the recovery of bromine is approximately 3 to 7% below the theoretical, this error is comparatively low for work of this nature where the quantities to be determined are very small.

The precision of the test is indicated in Table I which shows the results of determinations on nine commercial samples of unknown bromate concentration.

Since the standard error of the mean of two determinations equals the standard error of a single determination divided by $\sqrt{2}$, the standard error of the difference between the means of two sets of duplicate determinations equals the standard error ($\text{S.E.}/\sqrt{2} \times \sqrt{2}$). Taking the level of significance as twice the standard error, a difference of 0.00019% KBrO_3 between the means of any two duplicate determinations is statistically significant.

The method is applicable in the presence of chlorates, iodates and persulphates. In the ashing, persulphates are decomposed while chlorates, bromates and iodates are reduced to the corresponding halides. By controlled oxidation with the chromic-sulphuric acid mixture only bromine is liberated. The specificity of the method is shown by the results given in Table II.

TABLE I
RESULTS OF DUPLICATE DETERMINATIONS ON COMMERCIAL SAMPLES

Reference number	Potassium bromate content	
	Mean	Difference between duplicates
	%	%
1	0.001682	0.000225
2	0.001274	0.000072
3	0.001562	0.000047
4	0.000914	0.000228
5	0.001114	0.000141
6	0.001582	0.000176
7	0.001694	0.000047
8	0.001569	0.000068
9	0.001703	0.000017
Mean	0.001455	0.000113
Standard error of single determination 0.000097		

TABLE II
EFFECT OF PRESENCE OF OTHER FLOUR IMPROVERS

Sample number	Improvers present	Concentration present	Concentration found as KBrO_3	Recovery
		%	%	%
1	Potassium bromate	0.004	0.003930	98.2
2	Potassium bromate	0.004	0.003815	95.4
	Potassium iodate	0.001		
3	Potassium bromate	0.004	0.003825	95.6
	Ammonium persulphate	0.002		
4	Potassium bromate	0.002	0.002020	101.0
	Potassium chlorate	0.002		
5	Potassium bromate	0.002	0.001935	96.8
	Potassium iodate	0.001		
	Ammonium persulphate	0.002		
	Potassium chlorate	0.002		

Summary

Experiments with Kulman's flotation method for separating added potassium bromate from treated wheat flours showed it to be quite unreliable, particularly for concentrations below 0.003%, the results being uniformly low with unsatisfactory replicability. Two colorimetric methods for the estimation of bromine described in the literature were also found to be unsatisfactory.

A method described by Yates for the micro-determination of bromine in blood, which involves ashing in the presence of potassium hydroxide and selective oxidation of the mixed halides with a chromic-sulphuric acid mixture of such concentration that bromide is completely oxidized to bromine, while the chloride remains unaffected and any iodide present is converted to iodate, was found to give good recoveries with potassium bromide solutions. The recovery was improved by

employing a special all-glass micro-reaction and absorbing vessel in which an additional potassium iodide absorber is provided.

Employing these modifications and working with bromated flours of known bromine content, leaching of the added bromate from the flours gives better recoveries than direct ashing.

The method, as finally developed for the estimation of bromine in bromated flours, is described. This involves leaching a 20-g. sample with water, ashing with KOH, selective oxidation of the bromide to bromine with chromic-sulphuric acid mixture in a special reaction and absorbing vessel, aeration and absorption of the bromine in potassium iodide solution and titration with 0.001N thiosulphate.

With flours containing 0.001% to 0.005% KBrO_3 the recoveries ranged from 93% to 97%. For a series of nine random duplicate determinations, the difference between duplicates varied from 0.000017% to 0.000228% KBrO_3 with a mean of 0.000113.

A difference of 0.000194% KBrO_3 between the means of duplicate determinations is statistically significant.

The results are unaffected by the presence of chlorates, iodates and persulphates in the treated flours.

Literature Cited

- American Association of Cereal Chemists.
1935 Cereal Laboratory Methods, 3rd Ed. Published by American Association of Cereal Chemists.
- Bobtelsky, M., and Rosowskaya-Rossienskaya, R.
1931 Contribution to the study of concentrated solutions of electrolytes. The susceptibility of halides to attack by means of chromic acid sulphuric acid in concentrated solutions of electrolytes. *Z. anorg. allgem. Chem.* **197**: 147-160. (Original not seen, *Chem. Abstracts* **25**: 3903.)
- Evans, B. S.
1931 New process for the determination of small amounts of bromide in chloride. *Analyst* **56**: 590-593.
- Francis, G. A., and Harvey, C. O.
1933 The micro-determination of bromine in blood. *Biochem. J.* **28**: 1545-1550.
- Kulman, J.
1934 A quantitative method for determining bromates in compounded flours. *Chem. Listy.* **28**: 241-242. (Original not seen, *Chem. Abstracts* **29**: 236, 1935.)
1934a Determination of bromate in flour. *Z. Untersuch. Lebensm.* **68**: 375-377. (Original not seen, *Chem. Abstracts* **29**: 1890, 1935.)
- Leipert, T., and Watzlawek, O.
1934 Micro-analytical determination of bromine in organic substances. *Z. anal. Chem.* **98**: 113-116.
- Meyer, A. W.
1931 The chemical analyses of some important baking ingredients. *Cereal Chem.* **8**: 482-495.
- Scott, W. W.
1925 Standard methods of chemical analysis. 4th Ed., Vol. I, p. 96, D. Van Nostrand Co., New York, N. Y.
- Stenger, V. A., and Kolthoff, I. M.
1935 Detection and estimation of bromide. *J. Amer. Chem. Soc.* **57**: 831-833.
- Tod, H.
1933 An improved colorimetric method for the determination of the bromide content of body fluids. *J. Mental Sci.* **79**: 373-375.
- Yates, E. D.
1933 The determination of bromine in blood. *Biochem. J.* **27**: 1763-1769.

STUDIES ON THE BAKING QUALITY OF FLOUR AS AFFECTED BY CERTAIN ENZYME ACTIONS. V. FURTHER STUDIES CONCERNING POTASSIUM BROMATE AND ENZYME ACTIVITY

J. W. READ AND L. W. HAAS

The W. E. Long Company, Chicago, Illinois

(Read at the Annual Meeting, May 1937.)

In a preceding paper (Read and Haas, 1937), we have reported the results of our studies dealing with the action of bromate toward certain proteases. The data presented in that paper did not provide any basis for concluding that bromate inhibits the action of the proteinase of wheat flour. Neither did bromate show any inhibitive action toward pepsin and trypsin or toward the protease contained in Taka diastase, but the activity of papain and bromelin was repressed. In this paper we shall present some further studies concerned with the same problem.

Brief Review of Literature

Reference has already been made in our preceding paper to some of the more important studies and these need not be reconsidered except in two or three instances.

Gortner and Sharp (1923) and Sharp, Gortner, and Johnson (1923) reported detailed studies of some of the colloidal properties of wheat flour as related to flour strength, and reached the conclusion that gluten quality is determined primarily by the colloidal condition of the gluten gel. Gortner (1927) found that the peptizable protein varied inversely with loaf volume. Harris (1932) studied the relation between total protein, peptizable protein and loaf volume. He obtained a high positive correlation between non-extracted protein and loaf volume.

Concerning processes involved in bread manufacture, Gortner (1931) stated that "the salts which are added play a role in the hydration of the gluten colloids and probably tend to tighten the gluten and increase its gas holding capacity. It is a matter of common knowledge among bakers that a saturated solution of calcium sulfate will 'tighten up' the gluten of a weak flour. Certain of the flour improvers which are upon the market probably owe their beneficial properties, at least in part, to their action upon the gluten colloids, in that they

increase the tenacity with which gluten particle adheres to gluten particle in the dough gel."

Gortner, Hoffman, and Sinclair (1929) concluded from a study of 12 wheat flours that there is great variability in the amount of protein that can be extracted from a given wheat flour by various salt solutions of equivalent ionic concentration. There is also an equally striking variability of the proteins of individual flours toward a single salt solution. They believed these differences to be associated with the colloidal properties of the wheat proteins, which may also vary because of heritable differences and environmental conditions.

James and Huber (1927) found that oxidizing agents greatly increased the limit of the extensibility and breaking strength of gluten. Rich (1934) concluded that the effect of artificial maturation and the action of "gluten improvers" such as bromate, must be of a colloidal nature.

Frey and Landis (1932) reported that sulfur dioxide, when present in extremely small amounts, produced a very marked effect on the colloidal properties of gluten. In connection with this statement Alexander (1932) pointed out that it is well known in the paper and the corn products industries that SO_2 exerts marked swelling and disintegrative action on protein-carbohydrate complexes.

Balls and Hale (1936) stated that "cyanides and sulphites, like cysteine and glutathione, are strong reducing agents. They are well-known activators of proteinases of the papain type. This activation results from the reduction of disulphide linkages in the enzyme to sulphydryl groups."

Experimental

The investigative studies to be presented in this paper will deal with the relation of bromic acid to pH, the adsorption of bromate by activated and inactivated enzymic preparations, the effect of sulphite on gluten, the saccharogenic activity of flour as influenced by the bromate and persulphate of potassium, the heat required to destroy the proteinase of malt, and the repressive action of an aqueous extract of soya bean on the activity of certain proteases. The technic employed has been sufficiently described in the preceding paper of this series. (Read and Haas, 1937.)

Relation of pH to Free Bromic Acid

The suggestion of Kosmin (1934) that bromic acid may be slowly set free in the dough batch and thus attack the proteinase contained in the flour would seem to be rather improbable. Bromic acid is a strong oxidizing agent and may be titrated iodometrically. It was considered

advisable to determine what pH is required in order to free bromic acid from bromate. The results of these tests have been recorded in Table I from which it will be noted that the hydrogen-ion concentration necessary for the presence of bromic acid is never reached in a normal dough.

TABLE I

BROMIC ACID FROM BROMATE AS RELATED TO pH
Citrate-HCl buffer mixtures were used to prepare 5% gelatin solutions of varying pH.
Temperature maintained at 30° C. in closed cabinet

pH	Titration after 4 hours	Additional titrations for the following 16 hours	Percentage of KBrO ₃ titratable over a 20- hour period
	<i>C.c. of standard thiosulphate</i>		
Control	7.00	0.00	100
1.9	7.00	0.00	100
2.9	1.40	2.40	54
3.5	0.35	0.90	18
3.9	0.05	0.30	5
4.5	0.00	0.00	None
4.9	0.00	0.00	None

With this information in mind, it was decided to titrate bromate against enzyme preparations before and after the destruction of protease, with a view to ascertaining to what extent the content of bromate might be affected by the presence of active protease. If the variation in titration for the active and the inactive enzymic preparations was definite and of recognizable magnitude, the destruction of enzyme by bromate might be indicated. These data are given in Table II. Here again the results appear to fall in line with those already

TABLE II

BROMATE ADSORPTION BY ENZYME PREPARATIONS

One hundred cubic centimeters of each enzyme preparation were digested with 50 mg. of bromate for 2 hours at 35° C. Then 25 c.c. of each preparation were titrated against 0.05 N thiosulphate.

Preparation	Distilled water controls	Protease active	Protease killed
	<i>C.c. standard thiosulphate</i>		
Merck's diastase (10% extract)	9.15	9.0	9.0
Raw pineapple juice ¹	9.15	8.4	8.7
Papain ¹ (6% extract)	9.15	8.4	8.4
Taka diastase (8% solution)	9.15	9.15	9.15
Pepsin (6% solution)	9.15	8.9	8.9
Trypsin (5% solution)	9.15	9.1	9.1

¹ Significant values appear to be indicated by the loss in bromate caused by papain and pineapple juice.

TABLE III

EFFECT OF SODIUM BISULPHITE ON GUM GLUTEN (HUNGARIAN MANUFACTURE) AS MEASURED BY WATER SOLUBLE NITROGEN ACCORDING TO THE PROCEDURE OF JORGENSEN FOR FLOUR. GLUTEN SAMPLES CONSISTED OF 25 G. EACH

Product	Control (autolytic)	Digested with 10 mg. of NaHSO ₃	Digested with 20 mg. of NaHSO ₃
<i>Mg. soluble nitrogen</i>			
Gum gluten as received	5.2	6.5	6.7
Same gluten heated in water suspension for 30 minutes at 90° C. to destroy protease	5.0	6.9	7.4

TABLE IV

HEAT REQUIRED TO DESTROY PROTEINASE IN MERCK'S DIASTASE OF MALT IN DRY STATE

Amount added	Tem- pera- ture	Controls unheated	6 hours at 110° C.	12 hours at 110° C.	12 hours at 110° C. 1 hour at 145° C.
<i>Mg.</i>	<i>° C.</i>	<i>Consistency of gelatin after 24 hr. at 27° C.</i>			
15	25	Very viscous	Very viscous	Very viscous	Firm
	0	liquid Hard	liquid Hard	liquid Hard	Hard
20	25	Viscous liquid	Viscous liquid	Viscous liquid	Firm
	0	Medium	Medium	Hard	Hard
30	25	Liquid	Liquid	Viscous liquid	Medium
	0	Soft	Soft	Medium	Hard
40	25	Liquid	Liquid	Liquid	Soft
	0	Very viscous liquid	Very viscous liquid	Soft	Hard
50	25	Liquid	Liquid	Liquid	Mobile
	0	Viscous liquid	Viscous liquid	Very soft	Hard
60	25	Liquid	Liquid	Liquid	Mobile
	0	Viscous liquid	Viscous liquid	Very viscous liquid	Hard
80	25	Liquid	Liquid	Liquid	Very viscous liquid
	0	Liquid	Liquid	Viscous liquid	Hard

TABLE V
 PROTEOLYTIC ACTIVITY AS AFFECTED BY A 10% AQUEOUS EXTRACT OF
 SOYA BEAN FLOUR¹

Quantity enzymic preparation added	Temperature	Papain (8% extract)		Raw pineapple juice	
		Controls	2 c.c. of soya bean extract	Controls	2 c.c. of soya bean extract
<i>Drops</i>	<i>° C.</i>	<i>Consistency of gelatin after 24 hours digestion at 27° C.</i>			
2	25	Liquid	Liquid	Liquid	Liquid
	0	Hard	Medium	Hard	Fairly hard
3	25	Liquid	Liquid	Liquid	Liquid
	0	Medium	Mobile, soft	Fairly hard	Medium
4	25	Liquid	Liquid	Liquid	Liquid
	0	Mobile, stiff	Viscous liquid	Medium	Soft
5	25	Liquid	Liquid	Liquid	Liquid
	0	Mobile, soft	Viscous liquid	Mobile	Mobile, soft
6	25	Liquid	Liquid	Liquid	Liquid
	0	Very viscous liquid	Liquid	Very viscous liquid	Viscous liquid

¹ An aqueous extract of soya bean contains a powerful fat per-oxidizing enzyme.

obtained by other methods of approach. The loss of titratable bromic acid is to be attributed apparently to its adsorption by, or to its oxidation of organic material other than protease, except possibly for papain and bromelin.

Action of Sodium Bisulphite on Gluten

The marked disintegrating action of sodium bisulphite on gluten is indicated by the results recorded in Table III. (See also Table II, paper IV of this series.) When investigated according to the procedure employed by Jørgensen for the estimation of water soluble nitrogen, the presence of the bisulphite increased the soluble nitrogen, and did so in some proportion to the quantity of salt present. One would thus be induced to conclude that sodium bisulphite is an activator for proteases. On the other hand, the data concerned with gelatin liquefaction would lead to just the opposite conclusion. Our results indicate definitely that the disintegration of gluten by means of the bisulphite is not to be attributed to the activation of protease.

Heat Destruction of Protease Activity in the Dry State

These data have been presented in Table IV. The enzymic product used was Merck's diastase of malt (U. S. P.—IX). The high proteolytic activity of this product makes it a suitable agent for study. In the dry state the proteinase of malt is shown to be surprisingly resistant to heat. This would indicate that the proteinase in flour would likewise be very stable towards heat.

Effects of an Aqueous Extract of Soya Bean Flour on Gelatin Proteolysis

The soya bean contains a very potent fat-peroxidizing enzyme which is water soluble. Some liquefaction tests were made with a 10% extract of soya bean flour to ascertain if such an extract contained any factor or factors which might affect the activity of several proteases. The results have been recorded in Tables V and VI. It is interesting to note that with the exception of papain and bromelin,

TABLE VI
PROTEOLYTIC ACTIVITY AS AFFECTED BY A 10% AQUEOUS EXTRACT OF SOYA BEAN FLOUR¹

Quantity enzymic preparation added	Temperature	Taka diastase (5% solution)		Trypsin (1% solution)	
		Controls	2 c.c. of soya bean extract	Controls	2 c.c. of soya bean extract
<i>Drops</i>	<i>° C.</i>	<i>Consistency of gelatin after 24 hours digestion at 27° C.</i>			
2	25	Liquid	Liquid	Liquid	Very soft
	0	Very soft	Medium	Soft	Hard
3	25	Liquid	Liquid	Liquid	Mobile
	0	Very viscous liquid	Medium	Very viscous liquid	Hard
4	25	Liquid	Liquid	Liquid	Mobile, soft
	0	Viscous liquid	Soft	Viscous liquid	Hard
5	25	Liquid	Liquid	Liquid	Mobile, very soft
	0	Liquid	Very viscous liquid	Liquid	Hard
6	25	Liquid	Liquid	Liquid	Very viscous liquid
	0	Liquid	Very viscous liquid	Liquid	Hard

¹ An aqueous extract of soya bean contains a powerful fat per-oxidizing enzyme.

the proteases investigated were repressed in their activity on gelatin. It may be added that Merck's diastase of malt was also inhibited to about the same extent as was pepsin. Trypsin appeared to be the most affected. It will be recalled from our preceding paper (1937) that in the case of bromate, papain and bromelin were the only proteases which were adversely affected, while in this instance they were the only proteases which were not adversely affected by the extract of soya bean.

Bromate and Diastasis

On the two flours investigated (Table VII) the effect of bromate was to increase diastasis. The results were consistently definite in this

TABLE VII
SACCHAROGENIC ACTIVITY AS INFLUENCED BY THE BROMATE AND
PERSULFATE OF POTASSIUM

Dosage per 5 g. of flour	Flour No. 153956	Flour No. 150004
Mg.	Mg. of anhydrous maltose per 10 g. of flour	
Controls (autolytic)	318	416
0.2, KBrO ₃	322	434
0.5, KBrO ₃	335	447
1.0, KBrO ₃	335	449
2.0, KBrO ₃	340	452
4.0, KBrO ₃	358	443
5.0, K ₂ S ₂ O ₈	321	—
10.0, K ₂ S ₂ O ₈	340	—

TABLE VIII
EFFECTS OF CERTAIN CHEMICAL TREATMENTS ON PROTEOLYTIC ACTIVITY¹
Merck's Diastase of Malt (10% aqueous extract)

Amount of ex- tract added	Tem- pera- ture	Controls	Quantity of chemical agents shown in milligrams					
			5 NaVO ₃	10 NaVO ₃	Controls pH, 1.9	5 BKrO ₃ pH, 1.9	Controls pH, 2.9	5 KBrO ₃ pH, 2.9
Drops	° C.	Consistency of gelatin after 24 hours digestion at 27° C.						
1	25	Liquid	Liquid	Liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	0	Soft	Soft	Soft	Hard	Hard	Hard	Hard
2	25	Liquid	Liquid	Liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	0	Mobile	Viscous	Viscous	Hard	Hard	Hard	Hard
3	25	Liquid	Liquid	Liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	0	Viscous	Liquid	Liquid	Hard	Hard	Hard	Hard
4	25	Liquid	Liquid	Liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	0	Liquid	Liquid	Liquid	Hard	Hard	Hard	Hard
5	25	Liquid	Liquid	Liquid	Very viscous	Very viscous	Very viscous	Very viscous
	0	Liquid	Liquid	Liquid	Hard	Hard	Hard	Hard

¹ It will be observed from this table that sodium meta-vanadate does not inhibit the activity of malt proteinase on gelatin, also that hydrogen-ion concentration of pH—1.9 and pH—2.9 practically prevents any hydrolysis of the gelatin.

direction, but were not always consistently uniform. It is probable that the stimulative action is not a direct one.

Influence of pH and Sodium Meta-Vanadate on Proteolysis

From Tables VIII and IX it will be observed that sodium metavanadate is not inhibitory toward the proteinase of malt or toward the proteinase of autolyzed yeast, also that enzyme activity is practically prevented by pH 1.9 and pH 2.9.

TABLE IX
EFFECTS OF CERTAIN CHEMICAL TREATMENTS ON THE PROTEOLYTIC ACTIVITY OF AUTOLYZED YEAST¹

Quantity of enzymic preparation added	Temperature	Mg. of chemical agent added to each flask			
		Enzymic controls	10 KBrO ₃	10 NaVO ₃	10 NaHSO ₃
<i>Drops</i>	<i>° C.</i>	<i>Consistency of gelatin after 24 hours digestion at 27° C.</i>			
5	25	Viscous liquid	Viscous liquid	Viscous liquid	Very viscous liquid
	0	Firm	Firm	Firm	Hard
8	25	Liquid	Liquid	Liquid	Viscous liquid
	0	Fairly firm	Fairly firm	Fairly firm	Hard
10	25	Liquid	Liquid	Liquid	Viscous liquid
	0	Fairly soft	Fairly soft	Mobile	Hard
12	25	Liquid	Liquid	Liquid	Liquid
	0	Mobile	Mobile	Mobile, soft	Very firm
15	25	Liquid	Liquid	Liquid	Liquid
	0	Mobile, soft	Mobile, soft	Mobile, very soft	Fairly firm
18	25	Liquid	Liquid	Liquid	Liquid
	0	Very viscous liquid	Very viscous liquid	Viscous liquid	Fairly soft
20	25	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	Viscous liquid	Liquid	Soft

¹ To obtain the enzymic preparation, yeast was autolyzed by the method described in British Patent No. 450, 529 (1936). The yeast was first plasmolyzed by the addition of from 5 to 10% of ethyl acetate. Water was then added and the pH adjusted to approximately 6.3 by adding a 5% solution of trisodium phosphate. Autolysis was allowed to proceed for 30 hours at 35° C. during which time the pH was maintained at approximately 6.3 by several separate additions of the phosphate solution. By this procedure, the original weight of yeast had been increased by approximately 150%. Material in suspension was readily removed by centrifuging.

On the basis of the investigative work thus far conducted, it seems to us that the problem of the nature of the action of bromate as a "dough improver" is one which demands further and more detailed study. We are unable to support the conclusions reached by Jørgensen and other investigators, namely, that the ameliorative action of bromate in flour dough is to be attributed to the inhibitory influence of the bromate on the proteinase contained in the flour.

Summary

The data recorded in Table I indicate that the pH required for the production of free bromic acid from bromate never occurs in the dough batch.

The disintegration of gluten by sodium bisulphite cannot be attributed to proteinase activation.

The liquefaction tests which are recorded in Table IV show that the proteinase of malt (in the dry state) is very stable toward high temperatures.

Bromate and persulphate of potassium, when present in suitable amounts, promoted saccharogenic activity.

An aqueous extract of soya bean contained some factor or factors which repressed the activity of trypsin, pepsin and the protease of Taka diastase, but did not inhibit the activity of papain and bromelin. Like trypsin and pepsin the proteinase of malt was also repressed.

Potassium bromate and sodium meta-vanadate exerted no inhibitive action on the protease obtained from autolyzed yeast (Table IX), but sodium bisulphite did inhibit the activity of yeast protease to a considerable degree. This behavior of the bisulphite is contradictory to certain statements which have appeared in recent literature.

Literature Cited

- Balls, A. K., and Hale, W. S.
1936 Further studies on the activity of proteinase in flour. *Cereal Chem.* 13: 656-664.
- Frey, C. N., and Landis, Quick.
1932 Colloidal aspects of baking chemistry. *Colloid Chemistry*, Vol. IV—Jerome Alexander, Chp. 34: p. 544—Chemical Catalog Co., New York.
- Gortner, R. A.
1927 Correlation of loaf volume with peptizing action of salts on wheat flour proteins. *Proc. Soc. Exptl. Biol. Med.* 24: 530.
- 1931 The colloid chemistry of wheat, wheat flour, and wheat flour products. *Colloid Chemistry*—Jerome Alexander, Vol. III, Chp. 42, p. 622—Chemical Catalog Co., New York.
- , and Sharp, P. F.
1923 The physico-chemical properties of strong and weak flours. III. Viscosity as a measure of hydration capacity and the relation of the hydrogen ion concentration to imbibition in different acids. IV. The influence of the ash of flours upon the viscosity of flour-in-water suspension. *J. Phys. Chem.* 27: 481-492 and 567-576.

- , Hoffman, W. F., and Sinclair, W. B.
1929 The peptization of wheat flour proteins by inorganic salt solutions. *Cereal Chem.* 6: 1-8.
- Harris, R. H.
1932 The relation between total protein, peptizable protein, and loaf volume as obtained by successive increments of potassium bromate. *Cereal Chem.* 9: 147-156.
- James, T. R., and Huber, L. X.
1927 A study of the physical properties of washed gluten. *Cereal Chem.* 4: 449-460.
- Kosmin, Natalie P.
1934 Kleberqualität und einige Faktoren, die diese beeinflussen. *Das Mühlenlaboratorium* 4: 110-115.
- Read, J. W., and Haas, L. W.
1937 Studies on the baking quality of flour as affected by certain enzyme actions. IV. Potassium bromate and enzyme activity. *Cereal Chem.* 14: 752-766.
- Rich, C. E.
1934 The chemical and physico-chemical changes induced in wheat flour by artificial maturing agents. *Cereal Chem.* 11: 201-227.
- Sharp, P. F., Gortner, R. A., and Johnson, A. H.
1923 The physico-chemical properties of strong and weak flours. VII. The physical state of the gluten as influencing the loaf volume. *J. Phys. Chem.* 27: 942-947.

WIDE-RANGE VOLUME-MEASURING APPARATUS FOR BREAD

WILLIAM H. CATHCART and L. CARROLL COLE

Research Department, American Institute of Baking, Chicago, Illinois.

(Received for publication May 27, 1937)

Included in the bread scoring method¹ of the American Institute of Baking is the volume of the loaf. The volume of the loaves received for scoring varies from 1,200 c.c. to 3,000 c.c. and the lengths from 8 inches to 14 inches. The determination of volumes over this wide range was a perplexing problem for some time.

Much experimental work has been carried on in order to find a piece of equipment that would measure with a fair degree of accuracy the volumes of loaves of this wide variation.

Preliminary Experiments

Preliminary experiments were carried out using the hour-glass type of device. Several modifications of this type are on the market. This type has been modified and adapted to small loaves by Geddes and Binnington (1928). Malloch and Cook (1930) have modified the design still further in order to increase the ease of construction and the accuracy of operation. Two possibilities of error were noted with this type of device:

First, the error introduced if the loaf is not perfectly flat on the bottom. In most of the hour-glass types described in the literature, the rape seed or material used is not able to get under the loaf, since the loaf rests on the bottom of the chamber which contains it. In some cases this error was found to be of considerable significance, as will be seen later.

Second, the error due to the enormous quantity of material used in the device. The apparatus must be of large enough construction to hold the largest loaf. Thus, it is apparent that when the volume of a small loaf is measured, an unusually large quantity of material must be used. This was found to introduce a considerable error even when the apparatus was recalibrated with a standard loaf of approximately the same volume. This undoubtedly is due to the variations in the degree of packing.

¹ As revised August, 1936.

An apparatus designed about ten years ago by the American Institute of Baking and listed by E. H. Sargent and Company overcomes the first error to some extent, since the loaf is more or less suspended in a large chamber. However, there are other disadvantages, especially the enormous quantity of material required to operate it and the small range of volumes which it will handle.

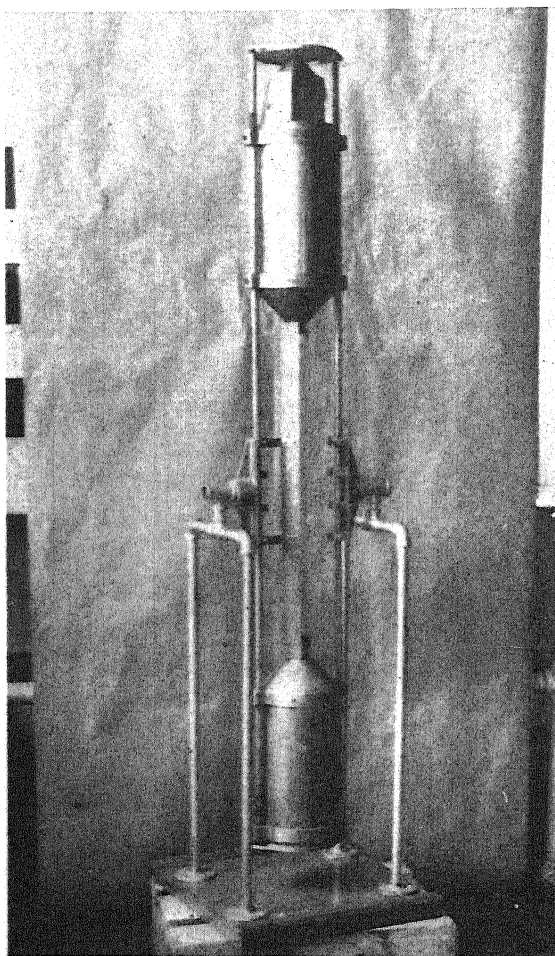


Figure 1a. The modified hour-glass type apparatus. The loaf of bread is held above the measuring chamber so that it may be seen.

The first error mentioned was overcome almost completely by a device similar in construction to those described by Geddes and Binnington, and also Malloch and Cook (Figures 1a and 1b). How-

ever, the method of suspending the loaf is different, it being suspended in the chamber on the thin steel rod *D* by means of the two aluminum washers *B* and the set screw *C*. This allows the material used to fill all of the space around the loaf except that covered by the two washers. This also has the advantage that it will accommodate sliced bread and handle loaves of varying lengths with the minimum amount of seed.

Although the apparatus shown in Figures 1*a* and 1*b* was designed to reduce the quantity of material used, the second error due to the variations in the degree of packing was still as great as ever.

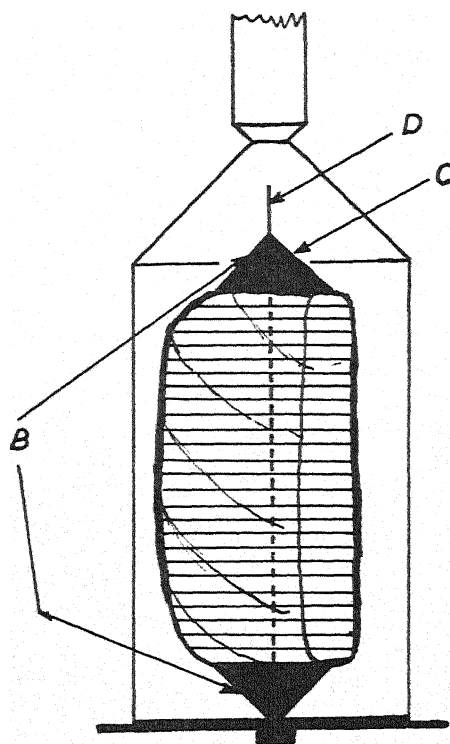


FIG. 1*b*.

In order to overcome the second error, a material was sought which would have practically no degree of packing. Many different materials were tried. Graduated cylinders were used and the degree of packing was measured. All of the seeds tried showed a large degree of packing. Of all the seeds tried, rape seed and rice gave the most reproducible results when allowed to flow freely through an orifice into the cylinder. Rice gave somewhat better checks than rape seed, but was more easily damaged. Static electricity, caused by the flow of the seeds over the

glass, seemed to be an important factor in the case of light materials such as flax seed and rape seed. Attempts to ground the cylinder were helpful but not satisfactory.

Glass beads ($1/8$ inch diameter at widest point) were better than the seeds, and aluminum balls ($1/8$ inch diameter) had hardly any degree of packing. However, when glass beads and the aluminum balls were used in the volume apparatus with various standard loaves, the errors were larger than with rape seed. It is felt this is due to the increased weight over that of the rape seed. For example, the error on various determinations of the same loaf was 0.4% for rape seed as compared to 0.8% for glass beads.

Since most inaccuracies enter from the material used, it is obvious that the smaller amount used the less will be the possible error. Thus, the following apparatus is the type that was finally adopted. It has been in use for eight months and has proved entirely satisfactory.

Other investigators (*e.g.*, Whitcomb, 1925) have mentioned trying similar devices; however, their procedure was different from that given below.

Description and Use of the Apparatus

The apparatus is shown in Figure 2 and is simply a box made of $1/2$ -inch thick plywood. The box is made with inside dimensions of 5 in. $\times$ 5 in. $\times$ 15 in. Blocks also of plywood were made to fit inside the box in three sizes—5 in. $\times$ 5 in. $\times$ 1 in., 5 in. $\times$ 5 in. $\times$ 2 in., and 5 in. $\times$ 5 in. $\times$ 3 in. The dimensions of the box are accurate to $1/32$ of an inch. With these blocks and others, if necessary, it is possible to adjust the volume of the box to almost any desired value. As many of the blocks are used as are necessary to leave just about $1/2$ inch space at each end of the loaf. A thin layer (about $1/2$ inch) of rape seed (or other material used) is placed on the bottom of the box, then the loaf whose volume is to be measured is placed in the box. Originally, the seed was allowed to flow into the box until full from an overhead hopper, which delivered a constant flow from a uniform height. The same degree of accuracy is accomplished, however, if the seeds are poured into the box through a wire screen with $1/4$ -inch openings.² The excess beads are scraped off the top, level with the box, by the use of a straight edge (the same must be used continually). Then the seeds around the loaf are poured off and weighed to the nearest $1/4$ ounce. This weighing introduces an error in most cases of less than 0.25% when glass beads or aluminum balls are used, and of less than 0.5% when rape seed is used.

² Experiments with a wire screen of this type and graduated cylinders showed that rape seed and glass beads gave a uniform degree of packing under these conditions.

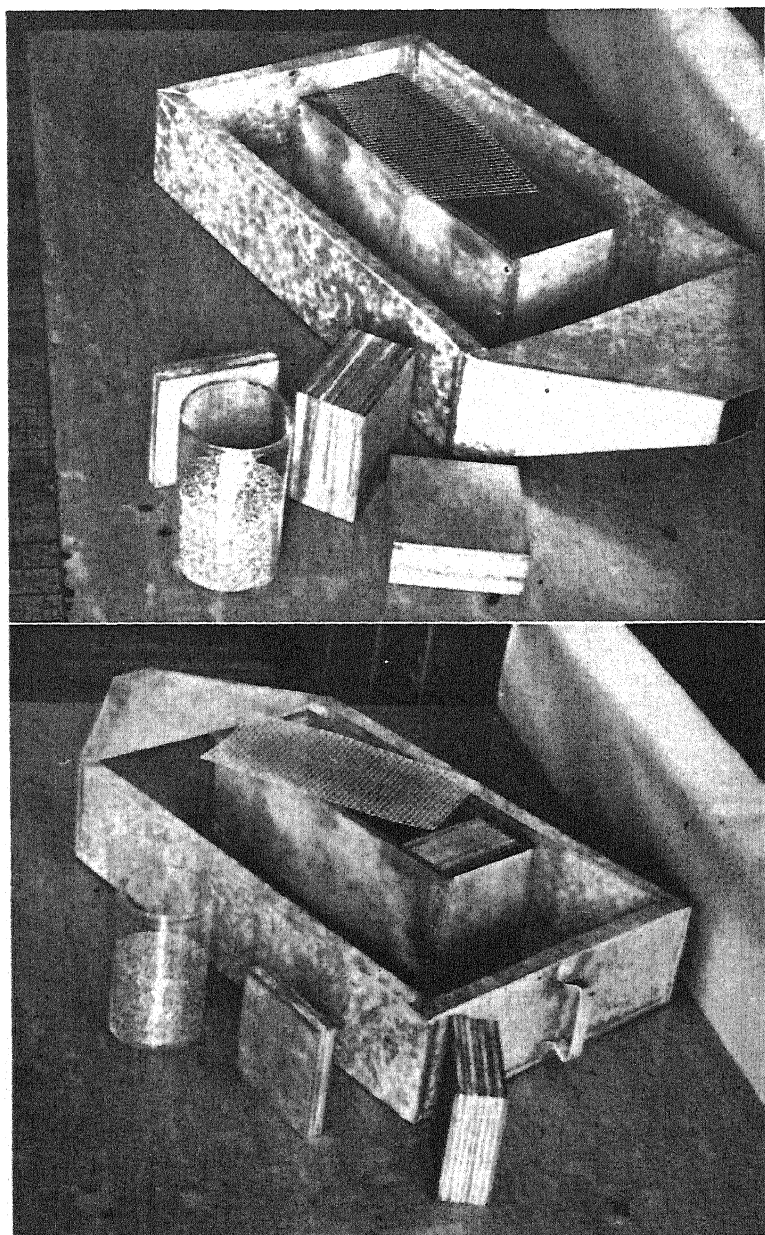


Figure 2. Wide-range-volume-measuring apparatus for bread. The top picture shows the empty box. The bottom picture shows the box with one block in place. The wire screen is set diagonally to show up in the picture. The blocks not in use and some of the beads are shown in both pictures.

By weighing the rape seed or other material used, it is possible to eliminate the large error due to the difference in the degree of packing that occurs in most other set-ups of this type, where the rape seed is measured in a graduated cylinder.

Experiments have shown that seed (or material used) pack differently in an empty box, in a box containing a loaf, and in a graduated cylinder. Where the cylinder is dispensed with and the rape seed weighed rather than measured, and the unit weight of seed determined by calibration using standard loaves, this error is reduced to a minimum. Due to this and tests with objects other than model loaves, the authors agree with Malloch and Cook that the use of water-filled balloons as recommended for calibration of loaf-measuring apparatus by Harrel (1928) is not satisfactory since they do not even remotely approach the shape of a loaf.

At first the arrangement used to catch the overflow seed and the seed from around the loaf was merely a cardboard box set on a slant, with a funnel-shaped end to allow the beads to run out into a can of tared weight. However, we now have a simpler construction from galvanized iron, as shown in Figure 2.

Knowing the volume of the box and the volume of the weight of the seed or other material used (by calibration), the volume of the loaf can be calculated.

For practical purposes the volume of the box can be taken as what the measurements indicate, that is, 5 in. $\times$ 5 in. $\times$ 15 in. = 375 cubic inches (or less, depending upon the blocks used in the box). The accuracy of the construction of the box is such that the volume taken in this way introduces an error within 1%. It is possible to decrease this error by determining the measurements of the box more accurately.

Calibration of Apparatus

The weight of a unit volume of seed (or material used) was determined by using one standard loaf and varying the volume of the box and by using three standard loaves and keeping the volume of the box constant.

The volumes of the standard loaves were determined by immersion in water. The values are given in Table I.

Loaves 1, 3 and 4 were coated with paraffin, as described by Malloch and Cook. The volumes of the loaves could be determined by this method with an accuracy of 0.1%.

The values in Table II were obtained with the standard loaves 1, 2 and 3, and the same procedure as described above under Description of Apparatus.

TABLE I
VOLUME OF STANDARD LOAVES
(Determined by immersion in water at 25° C.; accuracy 0.1%)

Loaf number	Description	Volume of loaf	
		In c.c.	In cu. in.
1	Bread, paraffin covered	1239	75.4
2	Aluminum model	1922	117.2
3	Bread, paraffin covered	2171	132.3
4	" "	2393	145.8

TABLE II
CALIBRATION OF MATERIAL
(Using loaves 1, 2 and 3; varying the volume of the box with loaf 2)

A	B	C	D	E	A - B	$\frac{A - B}{E}$	F
Volume box in cubic inches	Volume of standard loaf from Table I in cubic inches	Number of separate determinations	Variation in separate determinations in %	Weight of material to fill box with loaf. Average separate determinations in ounces	Volume of material in cubic inches	Cubic inches per ounce	Averages, cubic inches per ounce
Calibration of glass beads							
253.0	117.2	4	0.36	121.1	135.8	1.121	1.118
276.5	117.2	3	0.25	142.5	159.3	1.118	
301.0	117.2	2	0.18	165.6	183.8	1.110	
326.0	117.2	2	0.09	187.6	208.8	1.113	
276.5	75.4	2	0.27	180.4	201.1	1.113	
276.5	132.3	2	0.35	127.5	144.2	1.132	
Calibration of aluminum balls							
253.0	117.2	4	0.39	132.0	135.8	1.028	1.029
276.5	117.2	2	0.00	156.0	159.3	1.022	
276.5	132.3	2	0.48	139.1	144.2	1.037	
Calibration of rape seed							
253.0	117.2	2	1.49	50.6	135.8	2.68	2.68
276.5	117.2	2	1.49	59.4	159.3	2.68	
301.0	117.2	2	1.49	68.5	183.8	2.68	
350.0	117.2	2	0.56	86.8	232.8	2.68	
276.5	75.4	2	0.00	75.3	201.1	2.67	
276.5	132.3	2	0.37	53.6	144.2	2.69	

Column F of Table II gives the averages of the cubic inches per ounce for the various materials. This factor (cubic inches per ounce) multiplied by the weight of the respective material in ounces will give the corresponding volume.

Throughout this paper the volumes have been expressed in cubic inches. Cubic inches may be converted to cubic centimeters by multiplying by 16.4.

In Figure 3, the weight of material in ounces is plotted against the volume of material in cubic inches from Table II.

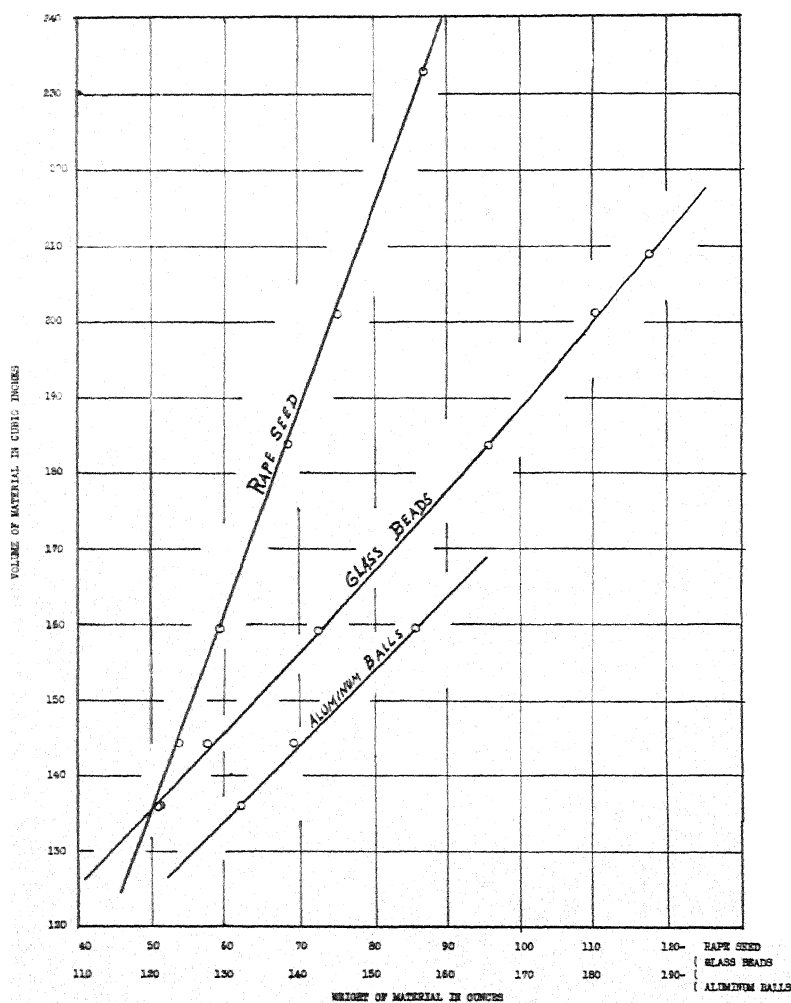


FIG. 3.

The loaf volume can be determined in two ways. First, by using the calibration curves of Figure 3, by means of which the volume of the material corresponding to its weight in ounces can be read directly.

TABLE III
 ACCURACY OF THE APPARATUS
 (Paraffin-covered loaves, numbers 1, 3 and 4, were used)

A	B	C	D	A - D	E	F
Volume of box in cubic inches	Volume of standard loaf from Table I in cubic inches	Weight of material to fill box with loaf in ounces	Volume of material from Figure 3 in cubic inches	Volume of loaf in cubic inches	Maximum error from volume in B column in %	Average error from volume in B column in %
Using glass beads						
253.0	75.4	160.00	177.9	75.1	0.53	0.27
253.0	75.4	159.75	177.7	75.3		
276.5	75.4	180.50	200.7	75.8		
276.5	75.4	180.75	201.0	75.5		
276.5	132.3	127.75	143.5	133.0	0.91	0.57
276.5	132.3	127.25	143.0	133.5		
301.0	132.3	150.50	167.8	133.2		
301.0	132.3	150.25	167.5	133.5		
301.0	145.8	140.00	156.3	144.7	0.75	0.48
301.0	145.8	139.25	155.5	145.5		
Using aluminum balls						
228.0	75.4	149.25	152.8	75.2	1.06	0.66
228.0	75.4	149.75	153.4	74.6		
276.5	132.3	139.50	143.2	133.3	1.28	1.00
276.5	132.3	138.75	142.5	134.0		
276.5	145.8	126.00	130.0	146.5	1.03	0.75
276.5	145.8	125.25	129.3	147.2		
301.0	145.8	150.50	154.3	146.7		
301.0	145.8	150.00	153.7	147.3		
Using rape seed						
253.0	75.4	66.25	177.8	75.2	2.27	1.23
253.0	75.4	66.75	179.3	73.7		
276.5	75.4	75.25	202.0	74.5		
276.5	75.4	75.25	202.0	74.5		
276.5	132.3	53.75	144.5	132.0	0.98	0.38
276.5	132.3	53.50	144.0	132.5		
301.0	132.3	62.50	168.0	133.0		
301.0	132.3	62.75	168.6	132.4		
301.0	132.3	63.25	170.0	131.0		
276.5	145.8	49.00	132.2	144.3	1.44	1.24
276.5	145.8	49.00	132.2	144.3		
301.0	145.8	58.50	157.3	143.7		
301.0	145.8	58.50	157.3	143.7		

Second, the average factors (cubic inches per ounce) given in column F of Table II may be used.

Accuracy of the Apparatus

In Table III the determinations of the volumes of the three paraffin-covered loaves (1, 3 and 4) are tabulated. The calibration curves given in Figure 3 were used. Loaves 1 and 3 were used in the calibration; however, it will be noted that other volumes of the box were used in this case. Loaf 4 was not used in any way in the calibration.

From Table III glass beads show a smaller percentage error than rape seed. Aluminum balls fall between glass beads and rape seed; however, not enough of them were available to make as many tests as was possible with the glass beads and rape seed. By using glass beads the maximum error is under 1%; however, when two or more determinations are averaged, the error is decreased to about 0.5%.

By recalculating all of the determinations of Table III, using the factors given in column F of Table II, it was found that the maximum error for glass beads was 1.5% and the average error 1%. The corresponding errors for aluminum balls and rape seed were about the same as those found in Table III.

The same tests as those given in Table II were repeated using glass beads only, but with no material having been placed in the box under the loaf. The average percentage error was 0.6 with loaf 1; 3.5 with loaf 3; and 2.2 with loaf 4. Thus, with loaves 3 and 4 large errors are introduced if material is not placed on the bottom of the box before the loaf is placed in it.

Advantages of the Apparatus

The apparatus described is cheap to construct. If it can not be made in the laboratory, a cabinet maker will make the whole thing for about five dollars. The overflow arrangement can be constructed out of galvanized iron by a tinsmith for an additional eight dollars; however, cardboard serves nearly as well. The most expensive item is the glass beads. They cost about seventy five cents per pound, and for the range of volumes with which we have to deal, 15 pounds are necessary. The glass beads, however, are washable; thus, they do not have to be replaced. They stand up very nicely in the apparatus and work well with sliced bread.³

³ The beads were obtained from the International Importing Bead and Novelty Company, 25 East Washington Street, Chicago, Illinois. When purchased, they were coated with a coloring matter, which must be removed before they are used. This can be accomplished by soaking for 24 hours in a strong soap solution or by means of an organic solvent such as benzene.

The accuracy of the apparatus, using a calibration curve over the range it is being used is less than 1%. If the volumes of the loaves which are to be determined vary only a small amount, the accuracy can be increased. In this case a box without any blocks can be used and the volume of the box determined quite accurately. This decreases the error due to the limit of accuracy in the construction of the box and blocks. The importance of this factor was pointed out by Harrel. This also means that approximately the same quantity of beads will be used all of the time. Experiments have shown that when the box method is used in this way the percentage error is less than with any of the hour-glass types tested.⁴

Summary

An innovation in the hour-glass type of apparatus is made.

A simple and quick apparatus for measuring loaf volume is described. The method of calibration, the way it is used, the accuracy, and the advantages of the apparatus are given.

The relative merits of various materials which can be used in the apparatus are discussed.

Acknowledgment

The authors wish to express their sincere appreciation to the Hubbard Portable Oven Company, Chicago, Illinois, for making the hour-glass type of apparatus; to the Hartford Steel Ball Company, Hartford, Connecticut, for lending the aluminum balls; and to Messrs. Morrill, Norton and Killen of the staff of the American Institute of Baking for helping with the preliminary work.

Literature Cited

- Geddes, W. F., and Binnington, D. S.
1928 A volume measuring device for small loaves. *Cereal Chem.* 5: 215-220.
Harrel, C. G.
1928 Calibration of loaf volume boxes. *Cereal Chem.* 5: 220-222.
Malloch, J. G., and Cook, W. H.
1930 A volume-measuring apparatus for small loaves. *Cereal Chem.* 7: 307-310.
Whitcomb, W. O.
1925 A study of methods of determining the loaf volume of bread. *Cereal Chem.* 2: 305-310.

⁴A box of any size can be constructed to meet any requirement of loaf volume.

A STUDY OF GLUTEN PROTEIN FRACTIONATION FROM SODIUM SALICYLATE SOLUTION. PART II. BREAD WHEAT GLUTEN FRACTIONATION

R. H. HARRIS

North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Received for publication July 3, 1937)

Introduction

In a recent paper the author (Harris, 1937) reported the results of an investigation into the gluten fractions of durum wheat flours, using the sodium salicylate dispersion method, followed by fractional precipitation of the dispersed gluten through the successive additions of concentrated MgSO_4 solution, as developed by McCalla and Rose (1935). In this study a significant positive relationship was found between the quantity of protein thrown down by 3 c.c. of the MgSO_4 solution and loaf volume. The quantity of wet crude gluten washed from the flours was also positively and significantly correlated with loaf volume, but the multiple correlation computed between the protein fractionated by 3 c.c. MgSO_4 and quantity of wet crude gluten with loaf volume was significantly higher than either of the total correlations. A gain of information in regard to loaf volume, or gluten strength, was thus obtained through a knowledge of the protein fractionated. In this instance loaf volume obtained by a short fermentation period of two hours and the incorporation of 5% of sucrose in the baking formula was considered to be a measure of the strength of the flour gluten. The loaf volume obtained by this method yielded a positive correlation of .9594 with the values obtained by the standard fermentation method. The quantities of protein removed by further fractionation did not have a relationship of any great importance with loaf volume. The initial protein fraction was not related to the crude protein content of the flours.

Harris and Bailey (1937) investigated the relation between the three thermal fractions of the gluten proteins employing the method worked out by Blish and Sandstedt (1933). These workers found the final or gliadin fraction to be positively correlated with loaf volume in the instance of 19 samples of *T. vulgare* wheats. The sodium salicylate method, however, did not involve the use of acid, alkali or alcohol which tend to denature protein, and appeared to be less cumbersome and laborious. In order to ascertain whether the fractionation of

gluten proteins from sodium salicylate dispersions by MgSO_4 solutions would yield the same information relative to loaf volume in the instance of hard red spring wheats as in the case of durum wheats, the following research was undertaken.

Experimental

MATERIAL AND METHODS

A 70% patent flour was milled from 30 samples of hard red spring wheat of the 1936 crop grown under various conditions of soil and climate. Wet crude gluten was washed from these flours according to the method followed by the author in the previous investigation cited, and recommended by Dill and Alsberg (1924). The washed gluten was dispersed in 10% sodium salicylate solution and fractionated into three portions by successive additions of 3, 8 and 10 c.c. of concentrated MgSO_4 solution. A fourth fraction remained in solution, and was not determined owing to difficulties mentioned in the previous paper. The fractions were removed from the residual solution by centrifuging approximately 20 minutes, and the quantity of protein separated determined by a modified form of the Kjeldahl-Gunning procedure. Foaming of the dispersions was prevented by the addition of a few drops of capryl alcohol. Moistures on the flours were determined in the air oven at $135^\circ \text{C.} \pm 2^\circ$ for one hour. Protein determinations were made on the wet crude gluten, and the gluten protein of the flour calculated therefrom. The gluten determination is not commonly used as a method for evaluating flour strength, but in the present instance it was necessary to obtain crude gluten for the purposes of fractionation and the extra time and labor involved in determining the gluten quantity and protein content were of no great importance. Ash was determined by the magnesium acetate method advocated by Bailey (1937) using 5 c.c. of acetate solution and an ashing time of one hour at approximately 700°C.

These flours were baked by the standard A. A. C. C. method, using 5% of sugar and a two-minute mix in the Hobart-Swanson. The loaf volume was considered to be a criterion of flour strength in the investigation, and no baking score was calculated for the flours. This attribute is subject to quantitative measurement, no question of individual judgment enters into its evaluation, and in this study the measurement of gluten strength alone was the paramount issue.

A further series of six flours was experimentally milled from a sample of hard red spring wheat, and various separations made corresponding roughly to different millstream flours produced in a commercial mill. The purpose of studying this set of flours was to learn, if possible, the quantity of protein removed as the first fraction by 3 c.c. of

MgSO₄ solution from flours of various baking strengths milled from the same wheat sample and presumably containing material originating in different portions of the wheat kernel. Harris (1931) found great variability in protein peptizability among millstream flours and attributed these differences to some extent to variation in the chemical constituents located in distinct parts of the berry. In the present instance only the first protein fraction was determined, as it has been found to be the one principally linked with loaf volume.

Discussion

The crude protein content of the wheat used in this study and the flours milled therefrom are shown in Table I, with ash, gluten content,

TABLE I
COMPARATIVE DATA ON FLOURS AND WHEAT PROTEIN (13.5% MOISTURE BASIS)
Results Arranged in Order of Increasing Loaf Volume

Sample number	Crude protein (N×5.7)		Ash	Wet crude gluten	Protein (N×5.7) in gluten	Gluten protein (N×5.7) in flour	Soluble protein per 100 c.c. solution	Loaf volume
	Wheat	Flour						
	%	%	%	%	%	%	Mg.	C.c.
37-1-74	16.3	13.9	0.38	40.5	28.7	11.6	436.0	319
81	16.4	14.9	.54	39.2	30.5	12.0	427.5	320
133	17.1	15.6	.53	44.0	28.8	12.7	416.1	342
80	16.3	14.7	.56	38.9	31.0	12.1	444.6	386
137	16.6	15.0	.50	42.5	30.6	13.0	438.9	418
68	16.7	14.1	.38	43.4	28.4	12.3	461.7	433
71	16.4	14.4	.36	44.2	28.5	12.6	521.6	458
77	15.4	13.4	.44	39.2	32.0	12.5	544.4	460
79	14.5	12.6	.50	38.9	29.5	11.5	510.2	469
78	15.4	13.0	.52	39.3	30.9	12.1	513.0	470
76	16.1	13.9	.39	41.6	30.7	12.8	538.7	472
72	19.5	16.6	.35	58.0	23.9	13.9	433.2	485
83	16.4	15.0	.50	43.1	30.3	13.1	481.7	488
73	16.8	14.8	.42	43.9	29.1	12.8	476.0	498
75	16.8	14.3	.27	48.2	27.8	13.4	478.8	500
62	18.2	15.6	.43	50.1	26.6	13.3	501.6	508
135	16.5	14.9	.40	41.4	30.5	12.6	461.7	515
64	16.9	14.9	.43	46.4	30.3	14.0	550.0	524
69	17.2	14.7	.36	44.5	29.2	13.0	644.1	534
65	17.5	15.4	.45	49.0	26.3	12.9	510.2	543
70	17.5	14.9	.52	47.7	28.9	13.8	541.5	544
115	17.4	15.3	.38	46.6	29.0	13.5	487.4	560
95	18.6	16.9	.50	52.2	28.1	14.7	473.1	575
37-2-9	15.8	13.3	.33	46.5	26.4	12.3	518.7	599
37-1-109	18.3	16.1	.42	48.6	29.1	14.1	487.4	610
37-2-3	16.7	14.1	.34	48.4	26.3	12.7	513.0	615
37-1-101	18.5	17.0	.54	52.3	27.8	14.5	478.8	616
37-2-2	16.1	13.5	.35	48.7	26.8	13.0	495.9	622
11	16.4	14.6	.37	49.9	27.5	13.7	521.6	670
10	16.4	14.0	.34	50.8	25.2	12.8	507.3	691

protein soluble in 10% sodium salicylate solution, and loaf volume. These values are arranged in order of increasing loaf volume, and cover a range of 372 c.c. from the smallest to the largest loaf. There does not appear to be any trend toward increase in wheat or flour protein with increase in loaf volume, but wet crude gluten and gluten protein do seem to increase in quantity in proceeding from top to bottom in this table, and the same trend is somewhat noticeable in the results showing the soluble protein. The variability in protein content among the wheats and flours is not very large, and certainly cannot account for the relatively large differences in loaf volume. This situation is somewhat different from previous researches conducted by the author on hard red spring wheat, when large variability existed in crude flour protein, these variations being closely associated with variability in loaf volume. The strength of this association appeared to be sufficient to justify the use of total flour protein as a means of forecasting flour strength from a knowledge of wheat or flour protein. The wet crude gluten values are somewhat more variable than the crude protein results. Large differences in the physical quality of the glutes were evident during the washing procedure, and it was quite easy to differentiate between the flours yielding satisfactory and poor loaves by the "feel" of the washed crude gluten. The glutes in the present study were not difficult to wash in contrast to a few of the durum wheat flours previously examined. The quantity of protein extracted by sodium salicylate varies to some extent among the various flours. The ash content of the flours ranges from 0.27 to 0.56% and this rather large difference is probably due in a large degree to the difficulty of milling such extremely hard and vitreous wheat as was produced in the 1936 crop season by exposure to abnormal growing conditions of drought and heat.

In Table II are shown the results obtained by fractionating the dispersed gluten protein through the addition of 3, 8 and 10 c.c. of concentrated MgSO_4 solution, the fractions thus obtained being designated as fractions 1, 2 and 3, respectively, for the sake of brevity. The total protein removed from solution by the combined additions of MgSO_4 is also shown, and represents the sum of the three fractions. It is evident that a considerable quantity of gluten protein remains in solution, and this quantity varies from sample to sample. A regular increase in protein thrown down as fraction 1 is noted with increasing loaf volume, but the other fractions do not show the same trend. This is in line with the work published by Harris (1937) dealing with similar studies on durum wheat flours. Great variability was found in the results obtained for the second and third fractions, and it did not seem possible to obtain the same precision in these cases as was achieved

TABLE II

PROTEIN (N X 5.7) FRACTIONS¹ PRECIPITATED FROM SODIUM SALICYLATE SOLUTION BY SUCCESSIVE ADDITIONS OF MgSO₄

Sample number	Fraction 1		Fraction 2		Fraction 3		Total protein removed from solution	
		Percent of soluble		Percent of soluble		Percent of soluble		Percent of soluble
	Mg.		Mg.		Mg.		Mg.	
37-1-74	66.7	15.3	233.2	53.5	19.4	4.4	319.3	73.2
81	89.5	20.9	207.4	48.5	44.4	10.4	341.3	79.8
133	78.7	18.9	230.3	55.3	57.6	13.7	366.6	88.0
80	75.2	16.9	226.2	50.9	46.8	10.5	348.2	78.3
137	86.6	19.7	253.1	57.7	51.3	11.7	391.0	89.1
68	68.4	14.8	207.0	44.8	79.6	17.2	355.0	76.8
71	120.3	23.1	260.0	49.8	64.4	12.3	444.7	85.2
77	112.3	20.6	238.8	43.9	67.2	12.3	418.3	76.8
79	120.3	23.6	220.0	43.1	67.2	13.2	407.5	79.9
78	123.1	24.0	225.8	44.0	61.0	11.9	409.9	79.9
76	130.0	24.1	247.4	45.9	64.4	11.9	441.8	82.0
72	80.9	18.7	297.6	68.7	68.4	15.7	446.9	103.1
83	126.0	26.1	233.6	48.5	48.4	10.0	408.0	84.7
73	92.9	19.5	306.6	64.4	10.2	2.1	409.7	86.0
75	107.7	22.5	174.4	36.4	13.2	2.7	295.3	61.7
62	92.3	18.4	238.8	47.6	81.0	16.1	412.1	82.1
135	108.9	23.6	236.5	51.2	55.9	12.1	401.3	86.9
64	117.4	21.3	252.6	45.9	70.2	12.8	440.2	80.0
69	132.8	20.6	311.8	48.4	74.1	11.5	518.7	80.5
65	109.4	21.4	240.0	47.0	72.4	14.2	421.8	82.6
70	130.5	24.1	245.6	45.4	67.2	12.4	443.3	81.9
115	139.6	28.6	241.1	49.4	59.3	12.2	440.0	90.2
95	137.4	29.0	232.0	49.0	51.8	10.9	421.2	88.9
37-2-9	132.8	25.6	265.6	51.2	55.8	10.7	454.2	87.5
37-1-109	137.4	28.2	245.1	50.2	61.6	12.6	444.1	91.1
37-2-3	107.2	20.9	244.0	47.6	71.8	14.0	423.0	82.5
37-1-101	126.0	26.3	242.8	50.7	54.2	11.3	423.0	88.3
37-2-2	114.0	23.0	244.6	49.3	72.4	14.6	431.0	86.9
11	155.0	29.7	227.4	43.6	69.6	13.3	452.0	86.6
10	161.9	31.9	207.0	40.8	64.4	12.7	433.3	85.4

¹ Fractions 1, 2 and 3 refer to quantities of protein precipitated by 3, 8 and 10 c.c., respectively, of MgSO₄ solution.

in the instance of the determination of the initial fraction. The maximum quantity of protein was removed by 8 c.c. of MgSO₄, and decreased sharply upon the addition of a further 10 c.c. of the reagent. No effort was made to calculate the total protein removed by each successive addition until the total 21 c.c. had been run in.

The correlation coefficients calculated between the various variables are shown in Table III, with corresponding tests of significance. Protein fraction 1 is significantly and positively correlated with loaf volume, this also being the case with percent of wet crude gluten and

TABLE III
CORRELATION COEFFICIENTS COMPUTED FROM THE FRACTIONATION AND
BAKING DATA

Variables correlated		r_{xy}	P^1
X	Y		
Loaf volume	Crude flour protein	+.0818	>.5534
Loaf volume	Wet crude gluten	+.6482	<.0002
Loaf volume	Gluten protein in flour %	+.5532	.0016
Loaf volume	Protein fraction 1 (mg.)	+.7769	<.0001
Loaf volume	Protein fraction 1 (% of soluble)	+.7277	<.0001
Loaf volume	Protein fraction 2 (mg.)	+.1098	>.5534
Loaf volume	Protein fraction 2 (% of soluble)	-.2544	.1786
Loaf volume	Protein fraction 3 (mg.)	+.3815	.0380
Loaf volume	Protein fraction 3 (% of soluble)	+.2278	.2258
Loaf volume	Total protein removed from solution (mg.)	+.6253	.0002
Loaf volume	Total protein in solution (mg.)	+.4616	.0104
Protein fraction 1 (mg.)	Wet crude gluten	+.2895	.1208
Crude flour protein	Wet crude gluten	+.3540	.0552
Crude flour protein	Gluten protein in flour %	+.6075	.0004
Total protein in solution (mg.)	Total protein removed from solution	+.7095	<.0002
Loaf volume	Wet crude gluten and protein fraction 1 (mg.)	r_{xy} +.8939	

¹ = probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.

gluten protein of the flour, while crude flour protein is quite unrelated to baking strength. The remaining fractions do not have a relationship of any great importance to baking strength. The total quantity of protein removed by $MgSO_4$ is, however, correlated with this variable, as is soluble protein to a lesser degree. The coefficient between crude flour protein and wet crude gluten approaches the horizon of significance, while the first variable and percent of gluten protein in the flour are more highly related. A significant positive correlation was found to exist between the quantity of protein dispersed by sodium salicylate solution and the total protein removed by the three portions of $MgSO_4$ solution added. The dispersed protein in milligrams is also positively related to loaf volume, and this finding agrees with the work reported by Sinclair and McCalla (1937) who postulated a decrease in gluten solubility in sodium salicylate in relation to baking strength when dealing with deteriorated flours.

A decided gain of information was obtained relative to loaf volume by fractionating the gluten protein, as is proved by the test of significance of the multiple correlation which measures the combined influence of wet crude gluten content and fraction 1 upon loaf volume. These latter data are shown in Table IV.

Comparative data between hard red spring and durum wheats are shown in Table V, the values for the durum varieties being taken from the study of durum wheat protein fractionation previously cited. The crude protein content of wheat and flour is higher for the durums, justifying the conclusion held by some that durum wheat tends to

TABLE IV

TESTS OF SIGNIFICANCE OF ADDITIONAL INFORMATION GAINED BY CALCULATING MULTIPLE CORRELATION COEFFICIENTS

Sum of squares	D F	Variance	F	5% Pt.
1 - (.6482) ²	28			
1 - (.8939) ²	27	.007442		
Difference	1	.378894	50.91	4.21
1 - (.7769) ²	28			
1 - (.8939) ²	27	.007442		
Difference	1	.195483	26.27	4.21

TABLE V

COMPARISON BETWEEN DURUM AND HARD RED SPRING WHEATS IN REGARD TO PROTEIN, ASH, AND GLUTEN FRACTION VALUES

	Crude protein (N×5.7)		Ash	Wet crude gluten	Gluten protein (N×5.7) in flour	Protein fractions ¹			Total protein re-moved	Soluble protein (N×5.7)
	Wheat	Flour				1	2	3		
	%	%	%	%	%	Mg.	Mg.	Mg.	Mg.	Mg.
Hard red spring	16.8	14.7	0.43	45.6	13.0	112.7	241.2	59.8	412.1	493.8
Durum	17.4	16.5	0.85	50.8	13.3	47.0	199.4	106.6	353.0	445.7

¹ Quantity of protein precipitated by 3, 8 and 10 c.c. of MgSO₄, respectively, from 100 c.c. of dispersion.

run higher in protein than common wheat varieties. The ash content is also much higher. The crude gluten content is higher in the durum but percent of gluten protein in the two classes of flour is almost identical. A comparison of the fractionation data for the two classes of wheat shows that the durum had a much smaller quantity removed as fraction 1, this difference decreasing with fraction 2, and being reversed in the instance of the third fraction. This result is in accord with the evidence presented by Harris and Bailey (1937) who found larger proportions of "gliadin" present in the non-*vulgare* wheat varieties. Presumably smaller protein particles exist in the final fractions removed by the procedure employed by Harris and Bailey and the method used in the present instance. Both total protein removed and protein solubility are lower in the case of the durum wheats as compared with hard red spring varieties.

Comparative data assembled from analyses of the six flours experimentally milled from a sample of hard red spring wheat of 16.6% protein to represent as closely as possible commercial "millstream" divisions are shown in Table VI. The differences in ash content of these flours point to the fact that materials from various portions of the wheat berry must have been incorporated in these classifications.

The flour from fifth break and tailings was extremely high in ash, and produced a loaf with the lowest color and loaf volume. This sample had also the highest crude protein and gluten content. Color was best in the instance of sample 2, which had good texture and the lowest ash. A rapid rise in ash occurred as the tail end of the process was approached, associated with a poorer crumb color. The crude protein increases in the same order, as is the case with wet crude gluten. Diastatic activity (Blish-Sandstedt) is at a minimum in sample 1, with a maximum being reached with sample 5, showing the inclusion of poor quality material with high enzymatic content in this flour. This sample also produced the poorest loaf. Moisture, of course, decreased with length of extraction and the associated longer time of exposure to the drying effect of the air.

In Table VII are given the fractionation and gluten protein values for the six flours. The protein removed by 3 c.c. of $MgSO_4$ solution was the sole fraction isolated, as it has previously been shown that

TABLE VI

COMPARATIVE DATA ON EXPERIMENTALLY MILLED FLOURS PRODUCED FROM ONE WHEAT SAMPLE AND CLASSIFIED TO REPRESENT DIFFERENT "MILLSTREAM" SEPARATIONS (13.5% MOISTURE BASIS)

Separation	Laboratory number	Crude protein (N $\times$ 5.7)	Moisture	Ash	Diastatic activity—maltose produced by 10 g. flour	Wet crude gluten	Loaf volume	Color	Texture
		%	%	%	Mg.	%	C.c.	Score	Score
1st sizings and 1st middlings	37-5-1	15.0	13.7	0.47	98.3	47.4	608	94	95
2nd, 3rd and 4th middlings	2	14.4	13.3	0.46	125.0	45.4	583	95	95
5th middlings, 1st tailings	3	15.8	12.0	0.68	170.0	50.2	612	94	95
2nd and 3rd break	4	17.8	13.7	0.72	130.0	56.5	670	93	92
5th break, tailings	5	20.3	11.3	1.52	229.0	66.9	555	86	93
1st and 4th break	6	19.2	13.3	0.82	126.7	66.1	624	92	94

TABLE VII

SOLUBLE PROTEIN, PROTEIN FRACTIONS AND GLUTEN PROTEIN IN FLOURS MILLED TO REPRESENT "MILLSTREAM" SEPARATION

Sample number	Soluble protein mg. for 100 c.c.	Protein fraction		Protein (N $\times$ 5.7) in gluten	Gluten protein (N $\times$ 5.7) in flour
			Percent of soluble		
		Mg.		%	%
37-5-1	501	146.6	29.3	29.3	13.9
2	507	161.8	31.9	28.2	12.8
3	501	155.8	31.1	27.9	14.0
4	508	165.2	32.5	28.2	15.9
5	456	119.6	26.2	24.4	16.3
6	499	154.2	30.9	26.3	17.4

further fractionation does not yield increased information in regard to flour strength. Considerable variation in protein solubility is evident, the best flours appearing to be the more easily dispersed in sodium salicylate, and the sample producing the largest loaf, sample 4, has the largest quantity of protein thrown down. On the other hand, the flour yielding the smallest loaf has the lowest quantity of protein removed by 3 c.c. of MgSO_4 solution. It would appear that the protein fractions vary among flours milled from the same wheat sample in much the same manner as among flours produced from various wheats. A similar conclusion was reached in regard to peptization by Harris (1937) in examining a series of commercially milled millstream flours produced from the same wheat blend.

The final picture of the gluten protein situation appears to the author at this stage as follows: wheat flour gluten may be reversibly fractionated into a large number of fractions which vary in appearance from a substance rather closely resembling the original gluten and which is easily separated into a hard, compact mass by centrifuging, to other constituents of a less viscous consistency which may be pulled into thin threadlike strands of great tenacity. It became more difficult to separate the later fractions as the protein adhered more tenaciously to any substance with which it came in contact. The initial protein removed appears to be rather highly related to flour strength in various types of wheat, and further work is programmed in these laboratories along this line of investigation. It is difficult to say whether the gluten proteins consist of one individual complex which may be split under appropriate treatment into a large number of fractions each differing in slight degree from its neighbor, or whether the fractions are thrown down as mixtures, each separation containing different proportions of the original constituent complexes. The author leans somewhat toward the latter viewpoint and is inclined to believe with Blish and Sandstedt (1933) that there are probably three distinct gluten proteins, or rather gluten complexes which differ among themselves in physical and chemical properties.

Summary and Conclusions

A series of 30 samples of flour experimentally milled to a 70% extraction from hard red spring wheat was analyzed for crude flour protein, ash, moisture, and wet crude gluten content. The gluten nitrogen was determined and the protein in the gluten computed. The flours were also baked by the standard A. A. C. C. method using 5% of sucrose. The glutens washed from the flours were then dispersed in 10% sodium salicylate solution, and fractionated by the successive additions of 3, 8 and 10 c.c. of MgSO_4 solution.

Fraction 1 was highly correlated with loaf volume, while the remaining two fractions had little relationship with flour strength. Crude flour protein was not related to loaf volume, while, on the other hand, percent of wet crude gluten and gluten protein were positively correlated with this variable. The same relationship was also apparent between total protein removed by MgSO_4 and loaf volume. A smaller correlation coefficient was found between loaf volume and the total amount of protein dispersed by sodium salicylate solution. The proportion of total protein removed from the salicylate dispersion appeared to vary with the solubility of the gluten in the dispersion medium.

A gain of information in respect to loaf volume was secured through determining the proportion of protein isolated by the initial volume of MgSO_4 added, over that furnished by a knowledge of wet crude gluten content.

A comparison between hard red spring and durum wheats showed the crude protein content of wheat and flour to be higher for the durums. The durum as a class had a smaller quantity of protein in the first fraction. This difference was less for fraction 2 and in the instance of the final fraction the hard red spring varieties had less protein precipitated. Both total protein removed and protein solubility are lower in the case of the durum wheat as compared with hard red spring varieties.

Further work of a similar nature was undertaken on six flours milled from a sample of hard red spring wheat to approach as closely as feasible to commercial millstreams. Only the first protein fraction was isolated in this series. This fraction appeared to vary among the different flours, the sample baking into the best loaf having the largest quantity of protein precipitated, while the poorest quality flour had the smallest proportion of protein thrown down. The fractions from the other flour samples varied between the two limit values. The six flours described appeared to approach quite closely to corresponding commercial samples in their chemical characteristics.

Acknowledgment

The writer wishes to acknowledge his indebtedness to T. Sanderson, who performed the milling and baking tests included in this investigation.

Literature Cited

- Bailey, L. H.
1937 Some observations on methods of ashing cereal products. *Cereal Chem.* 14: 120-128.
Blish, M. J., and Sandstedt, R. M.
1933 A new characterization of the gluten proteins. *Cereal Chem.* 10: 359-366.

according to Krishnamurti and Svedberg (1930)) and each lot will possess different properties depending upon the method of manufacture. Moreover, it is extremely sensitive to electrolytes, temperature, concentration, etc. (Kraemer, 1926). This very sensitivity, however, becomes a valuable characteristic in the study of small concentrations of proteolytic enzymes, provided other factors can be adequately controlled. The unique property of gelatin by which inter-chain coordination is magnified in the formation of gels makes detection of small changes a feasible proposition.

The collagen proteins in their many derived forms have long been used qualitatively and semi-quantitatively for the determination of proteases. Numerous investigators have used the gelation property in particular. The difficulty of exactly controlling conditions and the lack of knowledge regarding the effect of variations in the environment have somewhat limited the usefulness of such procedures. In the method to be described great pains have been taken to control temperature, gelatin and electrolyte concentrations and pretreatment of the substrate, which have resulted in a procedure that can be reproduced from day to day with confidence.

Gelation Rate Characteristics

It has been found that the rate of gelation, as indicated by increase with time of the viscosity, is susceptible to measurement and is directly related to the changes produced by proteolytic enzymes. Under the conditions of the experiment this function, *i.e.*, viscosity as a function of time of gelation, is given by

$$\frac{d\eta}{\eta dt} = a$$

or

$$\log \eta = at + b$$

and further, changes in this rate are, over a limited range, directly proportional to the concentration of certain proteolytic enzymes:

$$a = kc + a_0.$$

Here η is the apparent viscosity at time t , c is the concentration of enzyme and k a constant. The calculations are greatly simplified by the curious fact that the logarithmic gelation rate curves intersect at a common point not the origin after extrapolation. (Figure 1.). The reason for this unique behavior has not been fully elucidated.

The relationship between ionic strength and gelation rate has not yet been fully investigated, but it appears that at total electrolyte concentrations greater than 0.05 M the rate of change, while still

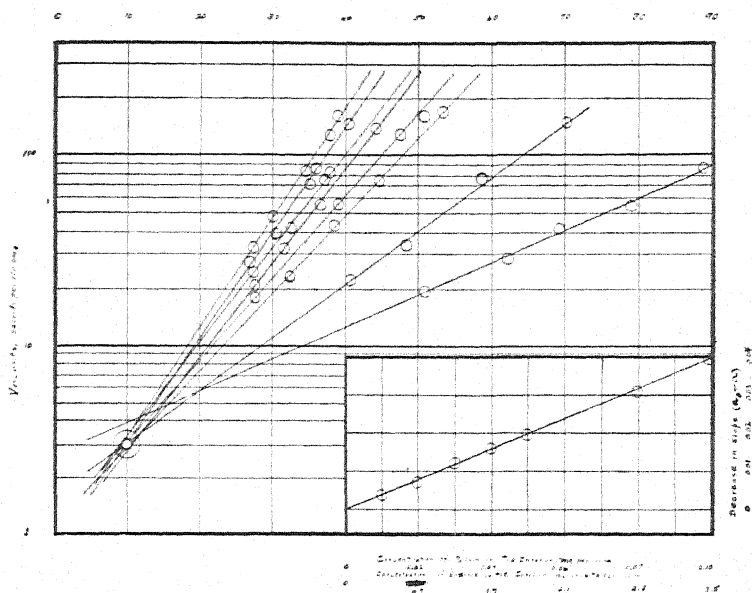


Figure 1. A typical calibration. In order of decreasing slope the curves represent the logarithmic gelation rates of a 0.75% gelatin solution after digestion for 20 hours with concentrations of papain equivalent to 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.08 and 0.10 mg. per 100 c.c. in the infusion. Extrapolation of each curve to the point of intersection gives the value of the pole, taken here as $\eta = 3$ seconds per 100 mm., $t = 10$ minutes. By the Gore (1929) method the activity of this sample of papain was found to be 35.0 units per gram. From these values the data represented in the lower right hand corner are obtained. The slope of this curve is 89.6 milliunits of activity per 100 c.c. of infusion per unit change in slope, which then becomes the calibration figure to be used for this procedure.

appreciable, is small. Hence a citrate-phosphate buffer at 0.06 M final concentration has been used. For the particular pork-skin gelatin adopted the pH-gelation rate curve has a maximum at pH 5.5 to 5.5 (Figure 2). The measurements are made within the range,

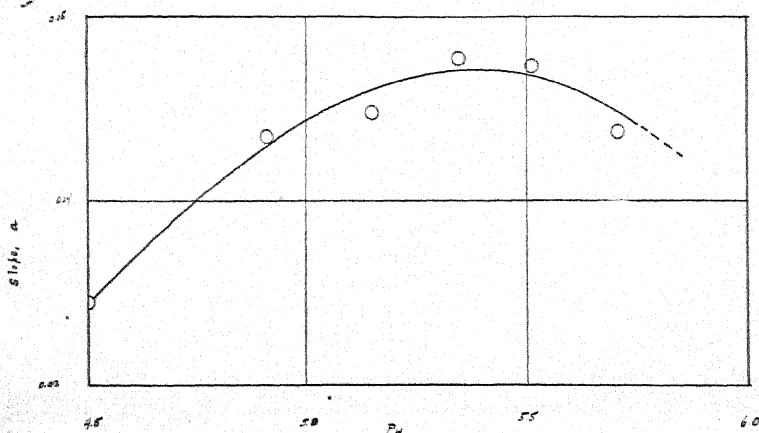


Figure 2. Effect of H-ion concentration on gelation rate.

which is the normal range of dough fermentation and not far removed from that existing in mashing practice.

The structure of a gelatin gel is commonly thought to be a complex brush-heap aggregation of micellar units. The growth of this structure is so markedly affected by temperature that slight irregularities are sufficient to produce a nonuniform gel in which inhomogeneities may be readily discerned. The structure is also very sensitive to mechanical shearing; once stirred or sheared the apparent viscosity of the gel decreases markedly. Fortunately, however, under the conditions to be described gelation proper is preceded by a stage of a-micronic aggregation, during which the solution exhibits true viscosity and is not susceptible to changes induced by shearing. If cooling of the solution during this stage is accompanied by gentle stirring, a sample without local inhomogeneities of temperature is obtained which will gel uniformly.

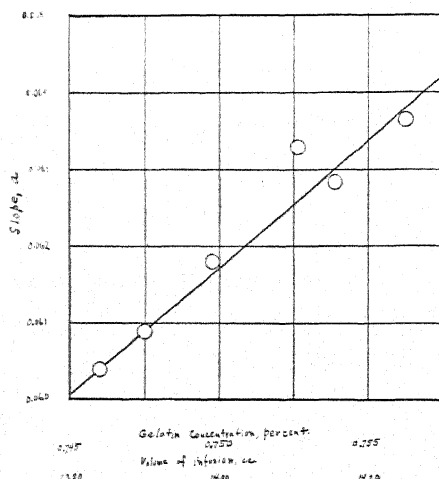


Figure 3. Effect of gelatin concentration on gelation rate.

It appears that gelatin, in common with a number of other such colloids, exhibits a temperature—degree of aggregation equilibrium. For example a gelatin sol prepared at a temperature below 40° C. when allowed to stand at 40° will gradually decrease in gelation rate (measured at 25° C.), while if prepared at 50° or higher it will gradually increase in gelation rate. Hence after preparation a certain interval must elapse before the use of the substrate in order that equilibrium may be approached.

Maintenance of a constant concentration of gelatin is of vital importance. The change in gelation rate produced by a small change in concentration over a small range is illustrated in Figure 3. It will

be seen that while the change is considerable it can be reduced to negligible proportions by using careful analytical precautions and keeping pipets well cleaned.

General Considerations

Since the reaction cannot be stopped without seriously impairing the gelation characteristics, it is necessary that the digestion period and testing time be so proportioned that the influence of the latter in the conversion measured is negligible. Partly to conform to this requirement and partly to increase the sensitivity, a digestion time of 20 hours at 30° C. has been adopted. As the testing is done at 15° C., where the reaction velocity is at least halved, and rarely requires more than 60 minutes, the amount of conversion occurring during testing must be less than 2.5% at most and is usually less than 1% of the total.

In view of the nonuniformity of gelatin and the lack of standardization of viscometers, thermostats and similar items of equipment, it is obvious that the method can hardly be put on an absolute basis. Consequently each unit of gelatin, each modification of procedure, must be calibrated in terms of a sample of known activity or of another method. We have used the method of Gore (1929) which, while lacking in sensitivity, depends on the changes induced in the mutarotation of gelatin, and is less dependent on the particular sample of gelatin used. Other methods applicable only to relatively active enzyme concentrates may also be used to calibrate the sensitive procedure.

Experimental

APPARATUS

Viscometer:—An eccentric falling ball type. Types in which any shearing of the gel may occur before test are unsuitable. One with interchangeable viscosity tubes is to be preferred, as the capacity is severely limited if only one tube is available. This viscometer should be completely submerged in the low temperature thermostat. A set of twenty matched viscosity tubes is a recommended accessory. Wall thickness should also be reasonably well matched.¹

Thermostat, high temperature:—A high temperature thermostat capable of regulating to within 0.05° C. This is set at 30° and is used for the 20-hour digestion.

Thermostat, low temperature:—A low temperature thermostat capable of regulating to within 0.001° C. and containing a device for

¹ Jena KPG precision bore tubing, 16.00 ± 0.01 mm. i.d., distributed by the Fish-Schurman Corporation, New York, has been used. A Hoppler viscometer ball 15.45 mm. in diameter appears to be suitable for these conditions.

rotating the viscosity tubes within the bath. This is normally set at 15° C. The construction of so-called ultra thermostats is a rather difficult and specialized task but it might be stated that cooling, heating, stirring and thermo-regulator lag must be properly balanced. Even then hunting is likely to occur if a heavy load of warm tubes is placed on the system.

Special pipets:—Calibrated to about one cubic centimeter less than half the capacity of the viscometer tubes. For KPG tubes 16.00 mm. i.d. by 6.63 inches long, uniformly closed by tightly fitting rubber stoppers, a pipet delivering 14 c.c. is suitable.

Clock, stopwatch:—Conventional analytical equipment.

REAGENTS

Distilled water:—Apply a vacuum of 5–8 mm. Hg to a sample of distilled water free from metallic contamination for several hours, not allowing the temperature of the water to drop much below room temperature. This removes most of the dissolved gases.

Buffer:—Dissolve 153.8 g. citric acid (0.8 mol) and 573.2 g. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (1.6 mol) in water, then make to a final weight of 4,370 g. with water. The pH should be between 5.0 and 5.5. After mixing separate into small samples of 200 c.c. each and sterilize in an Arnold.

Substrate:—Weight accurately into a beaker of convenient size the amount of gelatin which has been found by preliminary tests (see below) to give a convenient gelation rate (e.g., 3.75 g.—see Figure 3). Add not more than 10 times this weight of cold water (25 c.c.), stir, and after a thick paste containing no dry lumps of gelatin has been obtained transfer the whole to a double boiler (nested beakers, 50 c.c. and 100 c.c., respectively, are suitable). Stir carefully, avoiding foam formation, until a homogeneous solution is obtained (about 2 to 3 minutes are sufficient if not more than 5 g. of gelatin are being prepared). Pour into a flask containing 10 to 20 c.c. of hot water and rinse three times with hot water, using precautions customary to quantitative analysis. Add the required amount of buffer solution such that the concentration in the final reaction mixture will be 0.06–0.08 M (50 c.c. of buffer solution to 250 c.c. of substrate solution have been found satisfactory). Now make up with water to a final weight corresponding to that previously found to give a suitable gelation rate. It should be noted that this concentration is twice that existing in the final reaction mixture. Place in the bath at 30° C. to age for the required time (10 hours has been found satisfactory). This solution should now never be cooled below 30° C. until the final test for gelation

rate, else the equilibrium will be disturbed. It should be preserved under toluene.

Enzyme infusions:—Prepare enzyme infusions such that the final infusion contains from 1 to 3 milliunits per 100 c.c. For finely powdered materials of low activity which disperse easily, such as flours, drop the sample as a thin stream into a flask of water while rotating the flask. Allow to macerate for one hour, shaking at intervals. Then centrifuge and use the supernatant liquid for the determination, or a suitable aliquot thereof. For syrups disperse in the usual manner. For highly active powdered or coarsely ground concentrates such as papain weigh the sample (usually not less than 50 mg.) into a glass or agate mortar, triturate with a few drops of water, and wash quantitatively into a volumetric flask. Suitable concentrations are given in Table I.

TABLE I
RECOMMENDED CONCENTRATIONS

Material	Effective concentration in the infusion, per 100 c.c.
Wheat flour	3-5 g.
Dry malt extracts	3-5 g.
Liquid malt extracts	1-3 g.
Malt flours	0.01-0.2 g.
Animal digestive concentrates	1-50 mg.
Papain	0.05-2 mg.

PROCEDURE

The procedure cannot be described in absolute terms. For the substrate a large sample of gelatin should be obtained. A commercial food gelatin prepared from pork skin, moisture 10.0%, pH 4.5, isoelectric point 5.35 pH, has been used. It is first necessary to determine the concentration of gelatin required to give a satisfactory gelation rate. Following the procedure given for the preparation of the substrate various concentrations are investigated. It may be advisable to study both the pre-gel and gel stages, and a concentration selected which will give a common (Briggsian) logarithmic gelation rate of about 0.06 per minute. This choice is governed by the relative errors of determination of the time of fall of the ball in the viscometer and the precision of reading the time of gelation. Ordinarily the former is recorded to the nearest 0.2 second; the latter to the nearest 0.1 minute. Hence if the time of fall of the ball is regulated to be about 60 seconds (not over 200 centipoises, absolute measure) at 30 minutes the degree of precision of the two readings is of the same order of magnitude.

The general procedure in this determination (of suitable gelatin concentration) is as follows: An arbitrary concentration of say 0.75% gelatin is chosen for trial. Then 3.75 g. of gelatin are weighed out and made up finally with water to 250 g. including buffer as described above. After 10 hours at 30° C., 14 c.c. portions are transferred to a series of viscometer tubes, 14 c.c. of distilled water added, the contents mixed, and after 20 hours at 30° C. the testing of gelation rate is begun. The low temperature thermostat is adjusted to 15° ± 0.001° C. At regular intervals, say 2.5 minutes, the tubes are transferred one at a time from the 30° bath to the rotating device in the 15° bath, the time of transfer being recorded to the nearest 0.1 minute. After 10 minutes of rotation each tube is successively transferred to a stationary vertical position. Beginning at say 15 minutes after introduction into the 15° bath, the tubes are transferred successively to the viscometer and the viscosity determined at say 2- to 5-minute intervals, the time at which the viscosity test is begun being recorded to the nearest tenth minute, the time of fall being recorded to the nearest fifth second. The gelation time is then corrected to the mid-point of the viscosity determination. The data are then plotted on semi-log paper. It will be found that after a certain time, perhaps 25 minutes, the function becomes linear in $\log \eta$. In actual use measurements should be confined to this portion of the curve, the upper limit being that at which the viscosity determination becomes uncertain due to the great length of time required for the fall of the ball. The experiment is repeated with varying concentrations until a convenient value such as $\frac{d \log_{10} \eta}{dt} = 0.06 \text{ (min.}^{-1}\text{)}$ is obtained.

Having decided upon the concentration of substrate to use, the next step is to determine the pole, *i.e.*, the point of intersection of various gelation rate curves. (Figure 1.) A batch of substrate is prepared, aged, and 14 c.c. portions (or other suitable volume) pipetted into the viscometer tubes. Now 14 c.c. portions of enzyme infusions containing 0.1 milliunit increments of enzyme are added, the tubes stoppered, the contents thoroughly mixed, and digestion allowed to proceed for 20 hours. Enough replicates (at least two) of each concentration of enzyme should be included to determine each $\log \eta - t$ function. The solutions are then tested as described previously. It is convenient to determine the pole graphically by plotting η vs. t on semi-log paper. Since the extrapolation involved is considerable, an average value must be taken for the pole. This value is then used as one point on the curve in the calculation of subsequent results. For the conditions we have used the coordinates of the pole are $\eta = 3 \text{ sec./100 mm.}$, $t = 10 \text{ minutes}$.

CALIBRATION

Because the method is so highly empirical, each procedure must be calibrated. After the routine is fairly well established a standard sample of known activity may be run. The slope,

$$a = \frac{d\eta}{\eta dt} = \log \left(\frac{\eta}{\eta_p} \right) (1/(t - t_p)),$$

where η is the viscosity of the sample, η_p the viscosity coordinate of the pole, t is the corrected time of gelation and t_p the time coordinate of the pole, is calculated for the blank and for the active runs. The difference ($a_0 - a$) is plotted against the known concentration of enzyme units in the infusions. The slope of this curve gives the calibration of the procedure in terms of units of activity per unit change in the slope, $d\eta/\eta dt$. For the procedure here described this

TABLE II

TYPICAL SET OF DATA. DIGESTION TIME, 20 HOURS AT 30°. POLE: $\eta = 3$, $t = 10$

No.	Sample	Concentration in infusion g./100 c.c.	Gelation time, minutes				Viscosity, η , seconds		
			Start	Test	Time	Time corr.	Tube length, mm.	"Fallzeit"	
								obs. per 100 mm.	
1	Blank	0	810.0	838.9	28.9	29.2	100	39.2	39.2
2	Flour	6	812.5	894.7	82.2	82.6	50	48.2	96.4
3		4	817.6	877.8	60.2	60.6	50	47.2	94.4
4	"	3	820.0	864.5	44.5	44.9	100	47.8	47.8
5	"	2	827.5	870.4	42.9	43.2	50	41.2	82.4
6	"	1	830.0	868.7	38.7	39.1	50	46.4	92.8
7	"	0.05	832.6	866.8	34.2	34.7	100	62.2	62.2

CALCULATIONS ON THE PRECEDING DATA

No.	$\log (\eta/3)$	$\frac{\log (\eta/3)}{t - 10} = s^2$	$s_0 - s$	$90(s_0 - s) =$ milli- units per 100 c.c. of infusion	Proteolytic activity, milliunits per gram
1	1.116	$0.0582 - s_0$	—	—	— (blank)
2	1.507	0.0208	0.0374	3.36	0.57 ¹
3	1.498	0.0297	0.0285	2.56	0.64
4	1.202	0.0346	0.0236	2.15	0.71
5	1.439	0.0433	0.0149	1.34	0.67
6	1.491	0.0513	0.0069	0.62	0.62
7	1.317	0.0534	0.0048	0.43	0.86 ¹
Average					0.66

¹ Omitted from the average.

² s in the table = a in the text.

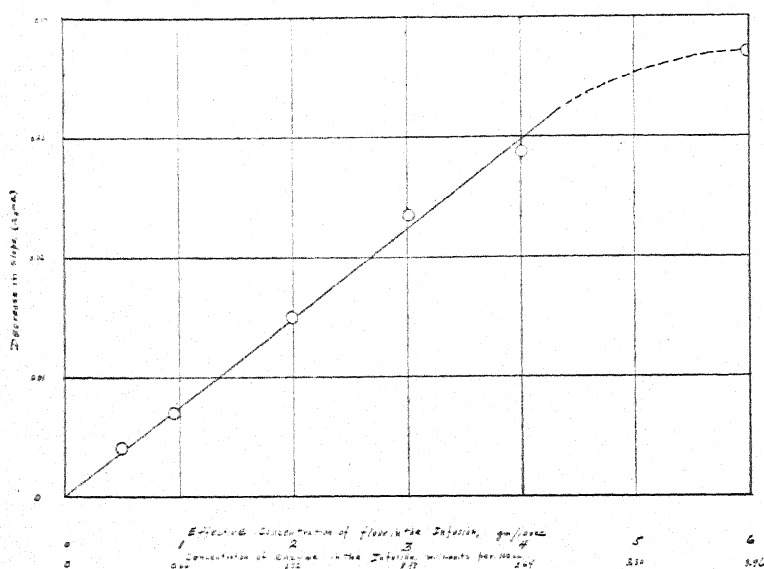


Figure 4. Allowable enzyme concentration range: Kansas flour.

TABLE III
SOME TYPICAL VALUES

Material used	Concentration g./100 c.c.	Activity, milliunits per gram
Flours		
N. W. short patent	3	0.43
N. W. high gluten	3	0.99
Kansas short patent	3	0.50
Texas patent	3	0.66
N. W. patent, unbleached	3	0.62
Same, bleached	3	0.41
Kansas, unbleached	3	0.61
Same, bleached	3	0.42
Soya flour	0.5	3.22
Barley malts		
6-day green malt	0.5	6.10
Pale Minn. Brewers'	0.5	5.03
2nd grade Vacuum Distillers	0.5	4.76
Malt extract, 1	1	2.34
Malt extract, 2	3	0.73
Malt extract, 3	3	0.20
Bakers' yeast		
Same, treated with CHCl_3 for 24 hours	0.2	12
Bacterial amylase	0.2	27
Pancreatic amylase	10^{-3}	1,400
Papain 1	10^{-4}	12,300
Papain 2	10^{-4}	45,700

value is 90 milliunits per 100 c.c. per unit change in slope, based on the Gore method (*loc. cit.*).

A typical set of data and calculations is given in Table II. This also illustrates the range within which enzyme concentrations must be kept. (Figure 4.) The values obtained for some common materials are given in Table III.

Summary

Dilute gelatin sols in equilibrium at 30° C., when cooled to 15° C., increase in viscosity, exhibiting true viscous flow before the onset of gelation proper. Gelation then proceeds according to the equation $\frac{d\eta}{\eta dt} = a$, where η is apparent viscosity at time t and a is a constant characteristic of the conditions. When digested with proteolytically active infusions a decreases, and over a limited range the decrease is directly proportional to enzyme concentration. This forms the basis of the sensitive method.

Literature Cited

- Bergmann, Max, and Rosa, Wm. F.
1936 Synthetic substrates for protein digesting enzymes. Jour. Am. Chem. Soc. 58: 1503- .
- Gore, H. C.
1929 Action of papain on the polarization of gelatin. Ind. Eng. Chem. Anal. Ed. 1: 203-208.
- Kraemer, Elmer O.
1926 Observations of the colloidal behavior of aqueous gelatin systems. Coll. Symp. Monograph 4: 102-121.
- Krishnamurti, K., and Svedberg, The
1930 The ultracentrifugal study of gelatin solutions. Jour. Am. Chem. Soc. 52: 2897-2906.

GERM CONTENT OF AMERICAN WHEATS ¹

C. H. BAILEY ²

Research Laboratories, General Mills, Inc., Minneapolis, Minn.

(Received for publication June 25, 1937)

Widely varying reports appear in the earlier literature respecting the percentage of germ or embryo in wheat kernels. Girard (1897), working with four French wheats, reported 1.16 to 1.50%; Fleurent (1896) found 1.50% in Algerian, 2.00% in Russian, and 2.05% in Canadian wheat; Osborne and Mendel (1919) reported 1.5%, presumably in American wheat; Percival (1921) recorded 2.8 to 3.5%; Grischenko (1935), of the Cereal Research Institute in Rostow/Don (U. S. S. R.), found 2.63 and 2.83% in two samples of the wheat variety Ukranka, and 2.56, 3.07, 2.81 and 3.25% in four samples of the variety Melanopus 069. Thus the percentages reported by Percival and Grischenko are considerably higher than the earlier findings. On the other hand Ingersoll and Bessey (1893) stated that the germ constituted 6% of the kernel, although the method of determination employed by them was not described and one is led to assume that they merely estimated the proportion from the apparent volume as seen by the microscope. Dedrick (1924) recorded the same estimate, possibly taken from Ingersoll and Bessey's statement.

It appeared desirable, in the light of these varying data, to ascertain the average of, and relative variations in the germ content of certain typical American wheats. To this end, several samples were collected by the wheat purchasing department of General Mills, Inc., which were regarded as representative of various types of hard spring, hard winter, and durum wheats. In addition, three samples of certain soft wheats were also secured.

There are no standard methods or techniques for the dissection of cereal kernels, and some preliminary experimentation was necessary to develop an acceptable procedure. After trying several methods the following was adopted for the purpose of this study: About 200 kernels, freed from broken and damaged grains, weed seeds and other foreign matter, were submerged for about five minutes in boiling water. They were then removed from the hot water, and allowed to stand for an additional 10 minutes. This treatment served to facilitate the subse-

¹ Paper No. 11, Journal Series, General Mills Research Laboratories.

² With the technical assistance of Catherine Becker.

quent detachment of the periderm over the surface of the germ and the later removal of the germ from the cavity in which it rests.

Two samples of 50 kernels each were then counted out at random. Using an eye surgeon's iris knife (see Figure 1), the periderm directly over the germ of each kernel was split, and peeled back. The germ was carefully cut loose at the edges. Gentle pressure on the starchy endosperm at the sides of the germ caused the embryo to slide out intact. The 50 germs thus separated from each sample were placed in

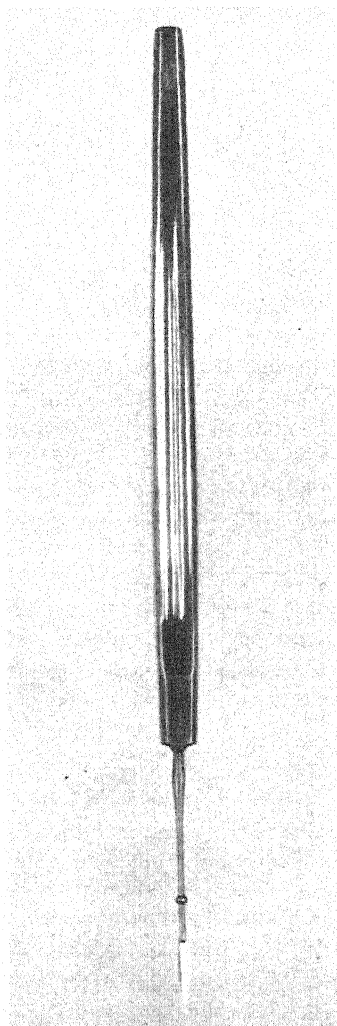


Figure 1. Iris knife used in wheat germ dissections (full size).

one vial, the residues from the same kernels in a second vial. These vials, unstoppered, were placed at once in an air oven maintained at 80° C. for several hours to dry them sufficiently so that spoilage would not occur. They were then corked and placed in a desiccator until a considerable number of dissections had been completed. The vials, with stoppers removed, were then placed in a vacuum oven and dried until they ceased to lose weight. They were then returned to the desiccator, stoppered, allowed to cool, and promptly weighed. The dried germs were weighed to the nearest 0.01 mg. on a micro-balance in the laboratory of the School of Chemistry of the University of Minnesota. This phase of the work was handled by a competent and specially trained micro-analyst. The dried kernel residues (ex-germ) were weighed on a high grade analytical balance to the nearest 0.1 mg.

The sum of the two weights was recorded as the moisture-free weight of the 50 entire wheat kernels, and these weights, divided by 50, were recorded as the average weight per kernel in milligrams. The average weight per germ in milligrams was likewise recorded, and this weight, divided by the weight per kernel and multiplied by 100, represented the percentage of germ. The averages of the two sub-samples taken from each wheat sample are recorded in Table I.

As a crude test of the accuracy of sampling and of germ separation it is interesting to note that the average difference in duplicate determinations was only 0.11% in terms of percentage of germ. This compares favorably with many chemical determinations in which the percentages involved are of the same order of magnitude.

From the data thus computed and recorded it appears that the percentages of germ in the hard red spring and hard red winter wheat samples examined by us are approximately the same. Moreover, they are in the same general range as reported by Fleurent for Canadian wheat (2.05%) and somewhat less than the findings of Percival and Grischenko. It thus appears that the estimate of Ingersoll and Bessey (6%) was substantially in error.

The variability was not large among the four spring wheat samples. We are at a loss to account for the low germ content of one sample of winter wheat, labeled "Turkey, Montana." The kernels in this sample were normal in shape and size, and did not differ widely from the average in kernel weight. In the instance of the other four hard winter wheats the variability was almost identical with that of the spring wheats, and was actually rather small. Moreover the hard winter wheats were very similar to the hard spring wheats in average germ content.

As a matter of added interest, two lots of kernels were separated by hand picking from the "Turkey, Montana" sample on the basis of

relative kernel size, and designated as "large" and "small" kernels. The germs were dissected from these two lots, and it developed that the percentage of germ was not greatly different in the instance of large and small kernels. Scanning the data resulting from the determinations on the hard wheats (spring and winter combined) indicates

TABLE I
GERM CONTENT OF HARD, SOFT AND DURUM WHEATS

Description of wheat and source	Average dry weight per kernel	Average dry weight per germ	Average germ
	Mg.	Mg.	%
<i>Hard Red Spring</i>			
Canadian No. 1 Hard, Ft. William, 1935 crop	25.81	0.582	2.26
Thatcher, University Farm, St. Paul, 1935 crop	20.88	0.509	2.44
Ceres, North Dakota, 1934 crop	27.37	0.603	2.21
Minnesota and North Dakota, 1935 crop	15.79	0.362	2.30
Average for Hard Red Spring	22.46	0.514	2.29
<i>Hard Red Winter</i>			
Oklahoma, 1936 crop	23.89	0.586	2.45
East North Central Texas, 1936 crop	23.58	0.522	2.21
No. 2 Hard, Nebraska, 1935 crop	18.74	0.412	2.20
Turkey, Montana	23.78	0.413	1.74
Large kernels	33.25*	0.578*	1.74*
Small kernels Separated from above	21.56*	0.400*	1.85*
Minturki, 1935 crop	21.73	0.500	2.30
Average for Hard Red Winter	22.34	0.487	2.18
Average for all hard wheats	22.39	0.499	2.23
<i>Durum</i>			
Mindum, St. Paul, Minnesota	32.12	0.895	2.79
Canadian, 1934 crop	32.25	1.033	3.20
Canadian, elevator mixture	29.40	0.915	3.11
Canadian	20.18	0.543	2.69
Canadian, 1935 crop	19.02	0.516	2.71
North Dakota	26.48	0.714	2.70
Elevator lot	27.09	0.742	2.74
Kubanka, Crookston, Minnesota, 1934 or 1935 crop	32.70	1.088	3.33
Average for Durum wheats	27.40	0.806	2.94
<i>Soft Wheats</i>			
No. 2 Soft White, 59.7 lbs., Spokane, Washington, 1934 crop	35.14	1.002	2.85
Soft Red Winter, Illinois, Indiana, Ohio, 1935 crop	24.97	0.593	2.37
Hybrid No. 128, Pullman, Washington, 59.5 lbs.	23.67	0.632	2.67
Average for soft wheats	27.93	0.742	2.66
Average for all wheats	25.30	0.662	2.61

* Omitted from average.

that there is no direct relationship between kernel weight and percentage of germ. This was further supported by computing the coefficient of correlation of these two variables which was $r = -0.184 \pm 0.149$. The correlation is small, and moreover is only slightly more than the probable error which renders it of doubtful significance. It is recog-

nized, to be sure, that the number of entries (nine) is too small for reliable computation in such instances.

In the case of the eight durum samples there is an indication of a positive relationship between kernel weight and percentage of germ, however, and the value of $r = +0.709 \pm 0.185$ gives evidence of a trend toward a larger germ in the heavier grains. The number of comparisons is too small, however, to establish this as a general principle.

It does appear that the durum germs are substantially larger than those of the *vulgare* (spring and winter wheats), and also constitute a larger percentage of the total kernel. The average of the three soft wheats was intermediate between the *vulgare* and durum, but they were so variable, ranging from 2.37% to 2.85%, and the numbers are so few as to make detailed comparisons impracticable.

Summary

Vulgare wheat samples of the hard spring and hard winter classes were rather uniform in percentage of germ in their tissues, and averaged 2.23%.

Durum wheat kernels had substantially heavier germs and thus had a higher average germ content, namely 2.94%.

Soft wheats examined were approximately intermediate between the *vulgare* and winter wheats.

The average germ content of 20 wheat samples was 2.67%.

Literature Cited

- Dedrick, B. W.
1924 Practical Milling. Published by National Miller, Chicago, Illinois.
- Fleurent, M. E.
1896 Sur la composition immediate du gluten des cereales. Compt. rend. 123: 327-330.
- Girard, A.
1897 Recherches sur la composition des bles et sur leur analyse. Compt. rend. 124: 876-882.
- Grischenko, Anna P., von
1935 Das Verhältnis der Bestandteile des Weizenkornes und ihr Aschegehalt. Das Mühlenlab. 5: 181-187.
- Ingersoll, C. L., and Bessey, C. E.
1893 Wheat and some of its products. Ann. Rept. Neb. State Board of Agric. p. 274.
- Osborne, T. B., and Mendel, L. B.
1919 The nutritive value of the wheat kernel and its milling products.. J. Biol. Chem. 37: 557-601.
- Percival, John.
1921 The Wheat Plant. A Monograph. Published by E. P. Dutton & Co., New York, p. 7.

AN IMPROVED METHOD OF SUGAR DETERMINATION IN DIASTATIC ACTIVITY MEASUREMENTS¹

F. C. HILDEBRAND and B. A. McCLELLAN

Research Laboratories of General Mills, Inc., Minneapolis, Minnesota

(Read at the Annual Meeting, May 1937)

The study of factors affecting diastatic activity and the control of such activity have in recent years become of such interest to cereal chemists that considerable effort has been expended in the development of suitable methods of determination. It is not our purpose to present here an extended review of the various methods which have been and are now in common use. It is significant to note, however, the large number of methods available, indicating not only the importance of such procedures, but also that no one method is sufficiently free from objections to have become universally adopted.

Recently Blish and Sandstedt (1933) and Putnam, Blish, and Sandstedt (1935) presented a procedure for the determination of diastatic activity in which the measurement of sugar concentration is based on the micro blood sugar technique developed by Hagedorn and Jensen (1923). This procedure, dependent on measurement of the unreduced ferricyanide from a known excess of that reagent, has been widely adopted by cereal workers because it offers several advantages over the older copper reduction methods. Since that time, a number of studies, of which those of Miller and Van Slyke (1936) and Whitmoyer (1934) should be mentioned in particular, have appeared in which the authors have reported improvements on the original Hagedorn-Jensen ferricyanide method.

Of the various methods advocated, that proposed by Miller and Van Slyke (1936) appeared to be best suited to modification to meet the requirements of a diastatic activity determination. Their procedure may be briefly summarized as follows: To the solution of reducing sugar is added an alkaline ferricyanide reagent containing a comparatively large and not necessarily accurately measured excess of ferricyanide. This mixture is placed in a boiling water bath for 20 minutes, cooled, and washed into the titration vessel with dilute sulfuric acid. The ferrocyanide formed by the reducing action of the sugar is titrated directly with a standard ceric sulfate solution, using an inside oxidation-reduction indicator.

¹ Paper No. 9, Journal Series, General Mills Research Laboratories.

We have revised this method to make it applicable to diastatic activity determinations, and in so doing have included a few minor changes which offer several improvements. The procedure, described in complete detail below, involves no new theoretical principles, but rather represents a combination of the best features of several methods in one determination which is rapid, convenient and accurate.

Procedure

A. Reagents

1. Alkaline ferricyanide.

Forty grams $K_3Fe(CN)_6$, 100 g. $Na_2HPO_4 \cdot 12H_2O$, and 12 g. NaOH per liter of solution. These materials need not be weighed more accurately than ± 0.02 g. This reagent deteriorates slowly in the presence of light and preferably should be stored in a dark bottle.

2. Ceric sulfate.

Approximately 100 g. of $Ce(SO_4)_2$ ² placed in a 1-liter beaker with 35 c.c. of concentrated H_2SO_4 and 35 c.c. of H_2O . The mixture is heated and stirred with the addition of more water in small increments until nearly all of the $Ce(SO_4)_2$ is dissolved. It is then filtered, cooled, and made up to 1 liter. The solution is standardized against Mohr's salt as described below, and diluted to give a final concentration of exactly 0.176 N.

From this stock solution, 100 c.c. are diluted to 1 liter, with the inclusion of 10 c.c. of concentrated H_2SO_4 , to give the solution for titration. This reagent thus is 0.0176 N with respect to $Ce(SO_4)_2$ and approximately 0.50 N with respect to H_2SO_4 .

3. Dilute sulfuric acid.

One hundred cubic centimeters of concentrated H_2SO_4 diluted to 1 liter, giving a final concentration of 3.58 ± 0.05 N.

4. Indicator.

Approximately 100 mg. of Setopaline C³ dissolved in 100 c.c. of water.

² Commercial product purchased from G. Frederick Smith Chemical Co., Columbus, Ohio.

³ This dye, the chemical constitution of which has not been disclosed, may be obtained from Eimer and Amend, New York. The indicator has an oxidation-reduction potential of +1.1 volts at pH 1.0.

B. Standardization of ceric sulfate

It is convenient in the standardization of the ceric sulfate stock solution to use a Mohr's salt of high purity as a primary standard.⁴ A 0.10 N solution is prepared containing 39.21 g. $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ and 25 c.c. concentrated H_2SO_4 per liter. Fifty cubic centimeters are pipetted into a titration vessel, 25 c.c. of the dilute sulfuric acid solution and 10 drops of indicator are added, and the Fe^{++} titrated with the stock ceric sulfate solution. The color changes sharply from a bright yellow-green to a golden brown, easily recognized against a white background.

The normality of the ceric sulfate solution is calculated from the expression

$$N = \frac{\text{c.c. Mohr's salt}}{\text{c.c. Ce}(\text{SO}_4)_2} \times 0.1000.$$

With the volumes given above, this becomes

$$N = \frac{5.000}{\text{c.c. Ce}(\text{SO}_4)_2}.$$

From this preliminary standardization, the permanent stock solution is prepared by accurate dilution to a concentration of 0.176 N.

C. Determination of reducing sugars

A 5 c.c. aliquot of the flour-buffer digestion mixture, inactivated and clarified in the usual manner (Blish and Sandstedt, 1933), is accurately pipetted into a large test tube, 10 c.c. of the alkaline ferricyanide reagent added, and the tube placed in a boiling water bath for exactly 20 minutes. At the end of this time, the tube is rapidly cooled, either under running water or by placing it in a large bath of cold water for 2 or 3 minutes. The contents of the tube are then poured into a 125 c.c. Erlenmeyer flask or other suitable titration vessel, using 25 c.c. of the dilute sulfuric acid reagent in two portions to rinse the tube. Ten drops of Setopaline C indicator are added and titration with ceric sulfate carried out as described under the standardization procedure. To obtain the diastatic activity of the sample, expressed as milligrams maltose per 10 g., subtract the volume of ceric sulfate in cubic centimeters used in the blank from that of the sugar determination and multiply the difference by 10.

⁴A grade of Mohr's salt designated as "Analytical Reagent, Low Manganese" obtained from Mallinckrodt Chemical Co., St. Louis, has been found to be entirely satisfactory.

D. Blank determination

Since there may be small amounts of ferrocyanide present in the ferricyanide used in reagent 10 above, and since the reagent itself is somewhat subject to deterioration, it is well in precise work to correct for these sources of error. A blank determination should be carried out, using 5 c.c. of a mixture of 46 c.c. acetate buffer, 2 c.c. of sodium tungstate solution and 2 c.c. of 10% sulfuric acid to replace the 5 c.c. of filtrate from the digestion mixture. The titration value thus obtained, which should not exceed 0.5 to 0.7 c.c. of ceric sulfate, should be deducted from the volume of ceric sulfate used in the actual determination.

Discussion

Since the procedure described is entirely empirical, it is vital to follow exactly the conditions prescribed in order to obtain accurate results.

Reagents: Experiments have shown that the oxidation of sugar solutions by an alkaline ferricyanide reagent is, like other sugar oxidations (see Caldwell, Doebbeling, and Manian, 1936), appreciably affected by the pH of the reaction system. To minimize the effect of small variations in alkalinity, we have used NaOH and Na_2HPO_4 as the alkalizing agent rather than Na_2CO_3 , utilizing the buffering effect of the former system.

Addition of sulfuric acid in the preparation of the dilute ceric sulfate solution is necessary to prevent hydrolysis, with resultant formation of insoluble ceric hydroxide or hydrous ceric oxides. Under the conditions prescribed we have found that the titration solution is entirely stable over periods up to four months' time. We have been unable to detect any change in the concentration of the stock ceric sulfate solution over the same length of time.

The dilute sulfuric acid solution, if it is only to be used to acidify the reaction mixture before titration, need not be carefully made up. We have found it convenient, however, to use the same solution in the inactivation of the flour-buffer digestion mixtures, and accordingly are quoting the specifications for this reagent given by Putnam, Blish, and Sandstedt (1935).

The indicator deteriorates somewhat with age, and the end-point becomes less distinct. We have experienced no difficulty in recognizing the end-point when using indicator solutions three or four weeks old, but operators unfamiliar with the titration may at first find it advisable to make up a fresh indicator solution each week.

Standardization: Other methods may be employed for the standardization of the ceric sulfate solution (see Miller and Van Slyke, 1936).

We have, however, found the procedure described above to be simple and reliable.

Determination of reducing sugars: The volume of sugar solution taken for analysis may be reduced if the maltose content of the sample is above 700 mg. per 10 g. In such cases, the aliquot taken should always be diluted to 5 c.c. In the calculation of maltose from the volume of ceric sulfate used, the factor 10 should be replaced by the factor appropriate to the particular aliquot of sugar solution taken.

Accuracy and range of the method: The procedure described has been carefully checked against solutions containing known amounts of maltose. A sample of commercial maltose was found to have a reducing power equivalent to 92.1% anhydrous maltose when determined by

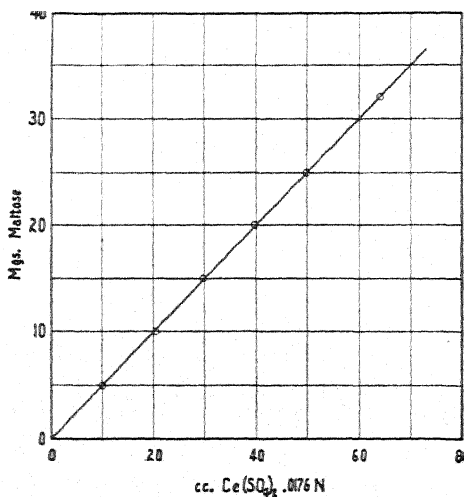


Fig. 1

the Quisumbing-Thomas copper reduction method. A known weight of this material was dissolved in a mixture of acetate buffer, sodium tungstate solution, and dilute sulfuric acid in the proportions present in an inactivated digestion mixture. Analyses of several such known maltose solutions, which thus reproduce the conditions of an actual diastatic activity determination, were made by the ceric sulfate method. The results, which are shown graphically in Figure 1, indicate that the relation between maltose concentration and ceric sulfate is a linear function up to a maltose concentration of 35 mg. per 5 c.c., which corresponds to a diastatic activity value of 700.

Comparative determinations by the Blish-Sandstedt and ceric sulfate methods were made on samples of six different flours. Aliquots

of the same digestion mixture were taken in the case of each flour, and determinations by each method were made in duplicate. The results, which are shown in Table I, indicate that the agreement between the two methods is excellent. Duplicate determinations by each method agreed to within approximately 0.5%.

TABLE I

COMPARATIVE DETERMINATION OF DIASTATIC ACTIVITY BY BLISH-SANDSTEDT AND $\text{Ce}(\text{SO}_4)_2$ METHODS

Flour number	Diastatic activity	
	Blish-Sandstedt	$\text{Ce}(\text{SO}_4)_2$
6400	182	182
6987	141	139
6990	213	211
6993	215	212
6997	290	293
6999	222	227

Advantages of the method: We believe the proposed ceric sulfate method offers substantial advantages in point of speed and convenience over the Blish-Sandstedt procedure. Since the range of maltose concentration which can be measured is nearly twice that of the Blish-Sandstedt method, the necessity for dilution of digestion mixtures is correspondingly less frequent. There is one less reagent to prepare and handle, and the preparation of the alkaline ferricyanide reagent has been much simplified, since there is no need for standardization. Because reduced ferricyanide is determined by direct titration rather than by difference, there is no necessity for extreme care in the amount of ferricyanide reagent added. The relation between maltose concentration and ceric sulfate used is a strictly linear function over the range 0 to 35 mg. of maltose per 5 c.c.; therefore a conversion table need not be used.

In the lower ranges of maltose concentration there seems to be little or no difference between the two methods as to accuracy and precision. Above a maltose concentration of 17 mg. per 5 c.c., the ceric sulfate method should be the more accurate, since the total maltose is determined directly rather than by dilution and the use of a multiplication factor.

Other applications: We have found the method entirely satisfactory in a number of determinations other than that of the amylase activity of flour. The activity of other amylase materials may, of course, be determined, and with suitable standardization other sugars may be determined as well. We have applied the method to the determination

of glucose with no difficulty. Despite the fact that Miller and Van Slyke report unsatisfactory results with fructose, we have been reasonably successful in the determination of invert sugar and of sucrose by inversion with this procedure. Preliminary results indicate that under the conditions specified, 1 c.c. of 0.0176 N ceric sulfate is equivalent to 0.466 mg. glucose and 0.462 invert sugar.

Summary

A modification, based on the micro sugar method of Miller and Van Slyke, of the Blish-Sandstedt diastatic activity procedure has been described. The method depends on the direct titration of ferrocyanide, formed by sugar reduction, with a standard ceric sulfate solution, using Setopaline C as an inside oxidation-reduction indicator.

The method proposed has a marked advantage over the Blish-Sandstedt procedure in point of time and convenience and also offers a substantial increase in accuracy with higher concentrations of maltose.

Literature Cited

- Blish, M. J., and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* 10: 189-202.
- Caldwell, M. L., Doebbeling, S. E., and Manian, S. H.
1936 Iodometric determination of maltose. *Ind. & Eng. Chem., Anal. Ed.* 8: 181-183.
- Hagedorn, H. C., and Jensen, B. N.
1923 Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. *Biochem. Z.* 135: 45-58.
- Miller, B. F., and Van Slyke, D. D.
1936 A direct microtitration method for blood sugar. *J. Biol. Chem.* 114: 583-595.
- Putnam, H. W., Blish, M. J., and Sandstedt, R. M.
1935 Factors affecting maltose values in the ferricyanide method for diastatic activity. *Cereal Chem.* 12: 494-504.
- Whitmoyer, R. C.
1934 Determination of small amounts of glucose, fructose, and invert sugar in absence and presence of sucrose. *Ind. & Eng. Chem., Anal. Ed.* 6: 268-271.

A SHORT GASSING POWER METHOD

R. M. SANDSTEDT

Department of Agricultural Chemistry, University of Nebraska, Lincoln, Nebraska

(Read at the Annual Meeting, May 1937)

Sandstedt and Blish (1934) have shown that in order to reduce yeast variability to a minimum in gassing power tests, it is necessary to use small quantities of yeast. This necessitates 4 hours to 6 hours for a gassing power determination. A reduction in the time required for the gassing power determination would be of great advantage in the mill control laboratory.

Blish and Sandstedt (1937) report that the rate of fermentation of maltose by fresh baker's compressed yeast can be increased enormously by the use of maltose fermentation activators. The most potent of these activators was prepared by drying "filler-free" (starch-free) baker's compressed yeast at low temperatures. A convenient method is to crumble the "filler-free" yeast, spread it over a flat surface, and dry it by use of an overhead heater and a current of air from a fan.

Preliminary experiments using this activator with fresh yeast showed that the rate of fermentation could be increased enough to shorten materially the time necessary for a gassing power determination, and that 0.5 g. of fresh yeast and 0.5 g. of yeast activator acting on a paste made with 10 g. of flour gave the most rapid fermentation with the minimum of error due to yeast variability. These quantities of ingredients gave results that could be checked from day to day in the same laboratory and gave concordant results between this laboratory and the Western Star Mill laboratory (Claude F. Davis).

Figure 1 shows typical gassing power curves of a series of flours using 0.3 g. of fresh yeast on a dough made of 10 g. of flour in the Sandstedt-Blish pressuremeter. Figure 2 shows the gassing power curves of this same series of flours using 0.5 g. of yeast and 0.5 g. of activator. It is seen that both methods give the same differentiation between flours and the same type of curve for each flour.

If the pressure readings are taken at the end of 2 hours and 3 hours, figures are obtained which indicate for most practical purposes the gassing-power potentialities of each flour. The 2-hour reading gives much the same information that is obtained from the combined maltose and sucrose values; *i.e.*, the quantity of readily available starch and sucrose.

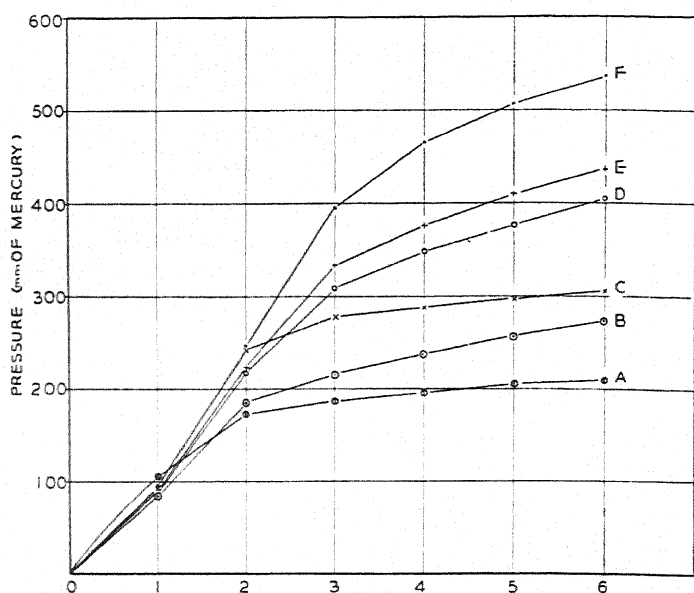


Figure 1. Gassing power curves of flour using 0.3 g. of yeast time (hours).

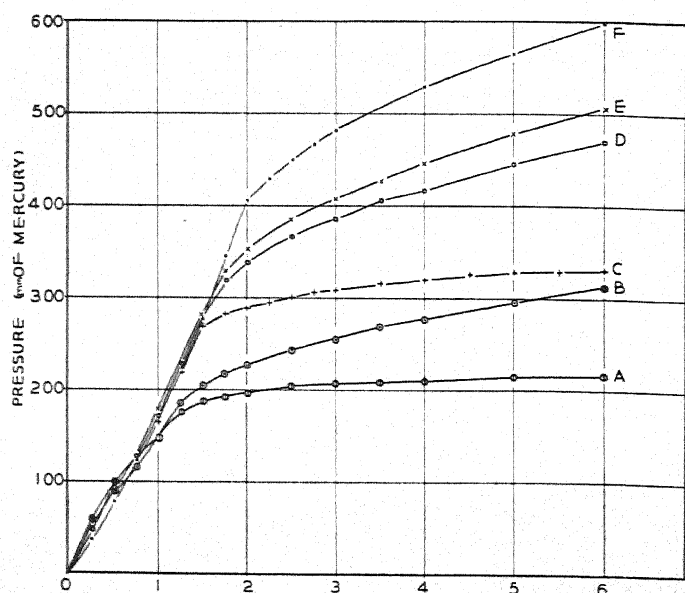


Figure 2. Gassing power curves of flours using 0.5 g. of yeast and 0.5 g. of activator time (hours).

The gain during the third hour gives a value indicative of the ability of the flour to sustain gas production over a long period. As may be seen from an examination of Figure 2, the reading at two hours is not always indicative of the ability of the flour to maintain gas production over a long period of time, hence the necessity for the 3-hour reading.

Adopted Procedure

The procedure as adopted is as follows: A suspension is made containing 0.5 g. of fresh baker's compressed yeast and 0.5 g. of dried "filler-free" yeast in 10 c.c. volume. This suspension is placed in the 30° C. water bath for one hour to ferment out the blank in the activator. A paste is made, in the pressuremeter, using 10 c.c. of the yeast activator suspension on 10 g. of flour. The procedure from the time of mixing the paste is the same as in the 4-hour to 6-hour method for determining gassing power (Sandstedt and Blish, 1934) except that the pressure readings are taken at the end of 2 hours and 3 hours.

Literature Cited

- Blish, M. J., and Sandstedt, R. M.
1937 Biocatalytic activators specific for the yeast fermentation of maltose. *J. Biol. Chem.* 118: 765-780.
Sandstedt, R. M., and Blish, M. J.
1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* 11: 368-383.

MECHANICAL SHAKING DEVICE¹

L. F. BORCHARDT, F. C. HILDEBRAND, and B. A. McCLELLAN

Research Laboratories, General Mills, Inc., Minneapolis, Minnesota

(Read at the Annual Meeting, May 1937)

In the course of various investigations in this laboratory, we have required a mechanical shaker (see Figure 1) which could be operated either continuously or intermittently in a constant temperature water bath. It was also desirable to have an apparatus sufficiently flexible in design to permit the use of flasks of several different sizes. We are here describing a shaker constructed to meet these requirements, hoping that others may find the information useful.

The main portion of the shaker, as will be seen from the accompanying illustration, is composed of a brass angle frame reinforced at the corners with brass blocks. Horizontally through these blocks are

¹ Paper No. 10, Journal Series, General Mills Research Laboratories.

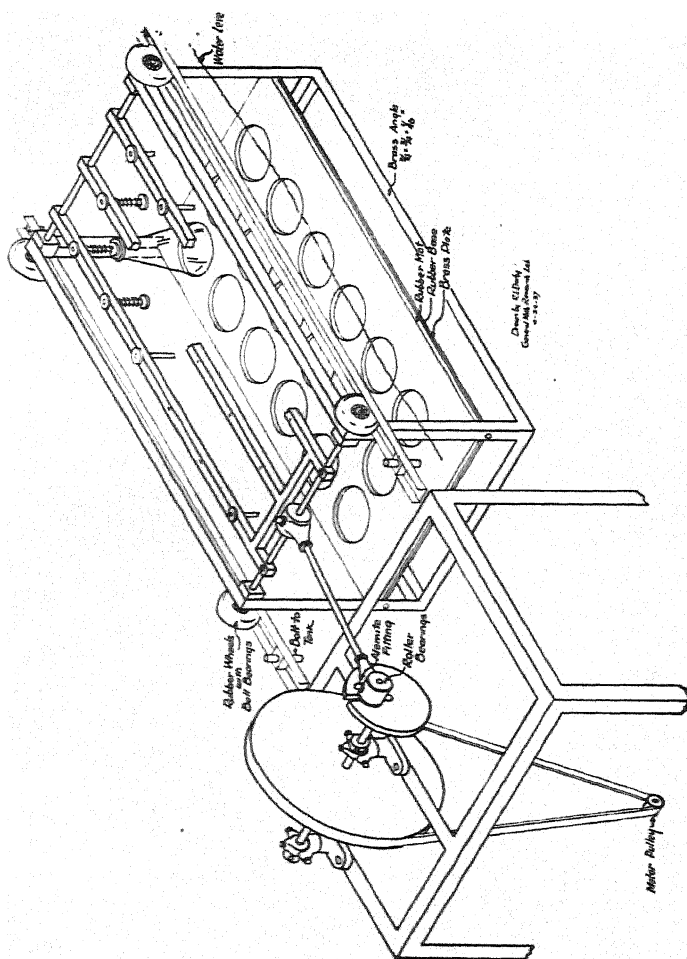


Fig. 1.

two steel axles on which are mounted ball bearing rubber wheels operating in channel iron tracks suspended over the constant temperature bath. Approximately three-quarters of the frame is below the surface of the water. The bottom, constructed of sheet brass covered with two layers of corrugated rubber matting, is bolted to the frame. It may be raised or lowered to accommodate varying sizes of flasks. Spring plungers passing through brass bars which parallel the long dimension of the frame hold the flasks in place by pressure on the stoppers. Additional security is given by cutting holes in the upper layer of rubber matting to fit the bases of the flasks.

The frame is driven by a connecting rod from the center of one axle to an eccentric, which in turn is mounted on a jack-shaft with a pulley of size sufficient to give approximately 100 r.p.m. The eccentric is slotted so that the distance from the connecting rod head to the shaft may be changed at will, thus varying the stroke of the frame. It has been found that with only a few flasks in the shaker there is a tendency to splashing, due to the formation of a standing wave in the water. This situation has been handled satisfactorily by placing an inverted brass trough around the inside of the bath to serve as a splash rail.

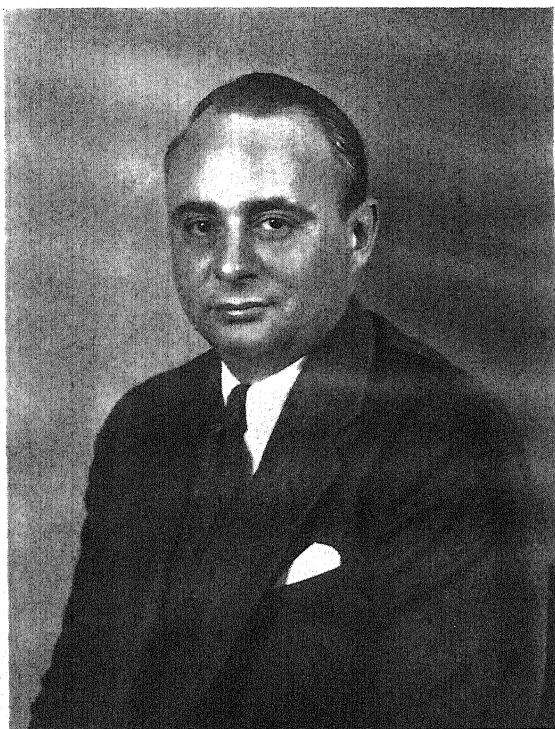
The shaker may be operated continuously as described, or intermittent agitation may be obtained by adding a regulating system constructed from a clock, a relay and a source of current (dry cells or small transformer). The clock is easily converted to a time switch. The moving contact is made by soldering a thin strip of spring brass to the minute hand, and the stationary points are of brass strips mounted on an insulated wire fastened to the clock face. The length of the stationary contacts will, of course, determine the time interval during which the shaker will operate. This time switch actuates the relay, which in turn opens and closes the circuit through the shaker motor.

This shaker has been in operation in our laboratory for nearly a year, and has given entirely satisfactory service.

We wish to thank R. I. Derby for preparing the drawing shown above.

DR. D. A. COLEMAN

1892 ~ 1938



Editor-in-chief of Cereal Chemistry

1931 ~ 1938

CEREAL CHEMISTRY

VOL. XV

MARCH, 1938

No. 2

A COMPARATIVE STUDY OF THE UTILITY OF VARIOUS COMMERCIALLY AVAILABLE ORGANIC SOLVENTS FOR THE DETERMINATION OF WHEAT AND FLOUR PIGMENTS¹

D. S. BINNINGTON,² L. D. SIBBITT,³ and W. F. GEDDES^{4, 5}

(Read at the Annual Meeting, May 1937)

Since the introduction of the "gasoline color value" test for flour pigmentation by Winton (1911), some form of petroleum hydrocarbon has comprised the solvent employed by the majority of investigators for the extraction of flour pigments. Winton (1911) and Coleman and Christie (1926) used "clear colorless gasoline" and Kent-Jones (1927) in a method employing two extractions specified "petrol" for the first, or carotenoid pigment fraction. Ferrari and Bailey (1929) in developing a spectrophotometric procedure, first employed petroleum ether but later (1929a) changed to high test gasoline. This solvent was again modified by Ferrari (1933) through the addition of 7% of absolute ethyl alcohol and the use of "light cleaners' naphtha" (boiling range 93.3° C. to 160° C.). Whiteside (1931) in an extensive study of the pigments of wheat and flour employed gasoline for the flour studies and a procedure involving pre-treatment with ammoniacal alcohol for the ground wheats. Geddes *et al.* (1934), in developing a simplified colorimetric method, used the naphtha-alcohol (93 to 7) solvent as suggested by Ferrari but employed a higher boiling naphtha (158° C. to 203° C.) known commercially as "varnish makers' naphtha" or "Stoddard's Solvent." Practically the only deviation from the use of petroleum hydrocarbons is that suggested by Simpson (1935), who in a double extraction procedure for determining the "yellowness" and "grade" of flours, employed 100% acetone as a solvent for the carotenoid fraction.

¹ Contribution from the Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba, with financial assistance from the National Research Council of Canada. Published as paper No. 125 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.

² Research Assistant, Associate Committee on Grain Research.

³ Technical Assistant, Associate Committee on Grain Research.

⁴ Chemist in Charge, Grain Research Laboratory, Board of Grain Commissioners for Canada.

⁵ The authors wish to acknowledge the assistance of H. Johansson and F. H. Lehnberg in securing many of the data presented. They are also indebted to C. G. Ferrari for many helpful suggestions in preparing the manuscript.

The requirements of an ideal solvent for the extraction of wheat and flour pigments may be summarized as follows:

High solvent power for the carotenoid pigments of wheat and flour.

Low solvent power for the chlorophyll and flavone pigments.

Ease of clarification, *i.e.*, capability of yielding brilliant extracts free from turbidity.

Uniform composition.

Moderate boiling point, low fire risk, non-toxic, and unobjectionable odor.

Insensitivity to atmospheric or sample moisture.

Low cost and ease of recovery.

On the basis of these criteria, petroleum distillates are far from ideal. They are not uniform in composition; the lower boiling members introduce errors by evaporation, tend to produce turbid extracts and increase the fire risk, while the higher boiling members have a wider distillation range and therefore a more variable composition which may be expected to affect the solvent properties. Turbidity of the extracts may be minimized and the solvent power increased by the addition of 7 to 10% absolute ethyl alcohol but this increases the cost and renders the reagent sensitive to atmospheric or sample moisture, tending to cause separation into two phases. The criticism regarding lack of uniformity in composition does not apply to a specific product or distillate produced according to rigid specifications by any one manufacturer. Workers in this field, however, can not conveniently secure their solvent from one producer and this would appear to be a source of variation in the results obtained by different laboratories.

In view of these considerations, the study reported here was undertaken with the object of securing a chemically homogeneous solvent which would in a large measure fulfil the requirements of an ideal solvent for the quantitative determination of wheat and flour pigments.

Experimental

An extensive range of organic chemicals of high purity is now available in commercial quantities and, accordingly, sixty of the most suitable were secured for a preliminary study. A large composite sample of experimentally milled, unbleached, hard red spring wheat flour was employed, the extracts being prepared and transmittancies determined by the spectrophotometric method outlined in the A. A. C. C. Book of Methods (1935). A Bausch and Lomb spectrophotometer equipped with a scale reading directly in transmittancy was employed. The results are presented in Table I, together with the carotene equivalents in parts per million of flour, boiling ranges, and

operating notes. It must be pointed out that the carotene values can only be considered as an approximate basis of comparison, due to the fact that they are computed from a transmittancy p.p.m. table based on the specific transmissive index ($K = 1.9145$) for pure carrot carotene in naphtha-alcohol at 4358 Å as reported by Johansson (1935). As the specific transmissive index varies with the solvent employed, it would be necessary to determine this value for each individual solvent in order to secure a precise relation between transmittancy and carotene. The preliminary nature of this phase of the study, however, did not justify the very considerable expenditure of time involved, particularly as a comparison of the transmittancies obtained is quite adequate.

A very wide variation in the apparent amount of pigment extracted will be noted, ranging from 0.17 p.p.m. for *n*-butyraldehyde to 2.62 p.p.m. in the instance of octyl alcohol. In general, the solvent action appears to be related to chemical constitution; this is shown by the means for each type of solvent illustrated graphically in Figure 1,

TABLE I

SUMMARY OF RESULTS OBTAINED IN INITIAL TESTS WITH VARIOUS SOLVENTS

Solvent	Transmittancy of extract (10 cm. cell)	Apparent carotene equivalent	Boiling point	Remarks
	%	P.p.m.	°C.	
<i>Hydrocarbons</i>				
Varnish Makers' Naphtha	21.98	1.72	150-180	Colour bleached out on standing.
Diamylene	28.20	1.44	150-170	
Benzene	23.14	1.66	79.6	
Toluene	21.84	1.72	110.7	
Xylene	12.18	2.38	144	
<i>Halogen Derivatives</i>				
Chloroform	17.00	2.01	60-62	Flour floats—necessary to filter.
Carbon tetrachloride	27.90	1.45	76.8	Flour floats—necessary to filter.
Ethylene dichloride (C.C.)	25.87	1.54	83.5	Flour floats—necessary to filter.
Ethylene dichloride (Dow)	22.61	1.68	83.7-84.0	Flour floats—necessary to filter.
Trichlorethane	19.44	1.86	74.1	Flour floats—necessary to filter.
Tetrachlorethane	22.80	1.68	146.3-147.0	Flour floats—necessary to filter.
Trichlorethylene	21.14	1.76	86.5-87.5	Flour floats—necessary to filter.
Tetrachlorethylene	27.72	1.45	121-122	Flour floats—necessary to filter.
Propylene dichloride (C.C.)	14.52	2.19	95.9	Flour floats—necessary to filter.
Propylene dichloride (Dow)	19.43	1.86	93-97	
<i>n</i> -Amyl chloride	20.55	1.79	102-110	
Mixed amyl chlorides	18.75	1.89	85-109	
Dichloropentanes	83.90	0.20	130-200	
α -Monochloronaphthalene	32.12	1.29	258	Flour floats—necessary to filter.
α -Monobromnaphthalene	40.70	1.02	281.1	Flour floats—necessary to filter.
<i>Alcohols (Primary)</i>				
Methyl alcohol (absolute)	—	—	64.5	Very opalescent; not read.
Ethyl alcohol (95%)	14.73	1.02	—	
Ethyl alcohol (absolute)	12.85	2.33	78.3	All alcohol extracts including primary, secondary and tertiary exhibit a tendency toward slight opalescence.
<i>n</i> -Butyl alcohol	12.78	2.33	117.9	
<i>n</i> -Butyl carbinol	13.23	2.30	134.5-138.5	
iso-Butyl carbinol	11.32	2.47	128.5-132.5	
sec-Butyl carbinol	11.82	2.42	125-131	
<i>n</i> -Hexyl alcohol	12.13	2.39	157	
2-Ethyl-butyl alcohol	14.37	2.20	148.9	
Octyl alcohol	9.92	2.62	184.6	

TABLE I—(Continued)

Solvent	Transmittancy of extract (10 cm. cell)	Apparent carotene equivalent	Boiling point	Remarks
	%	P.p.m.	°C.	
<i>Alcohols (Secondary)</i>				
Isopropyl alcohol	15.09	2.15	82.3	
Diethyl carbinol	14.27	2.21	99.8–103.8	
Methyl- <i>n</i> -propyl carbinol	13.04	2.32	117.5	
Secondary amyl alcohols	11.81	2.42	113–125	
Methyl amyl alcohol	13.09	2.32	131.4	
<i>Alcohols (Tertiary)</i>				
Dimethylethyl carbinol	10.56	2.54	99.8–103.8	
Tertiary amyl alcohol	14.33	2.21	98–111	
<i>Aldehydes</i>				
<i>n</i> -Butyraldehyde	86.0	0.17	71–78	
<i>Ethers and Oxides</i>				
Ethyl ether (absolute)	23.09	1.66	34.5	
Isopropyl ether	17.03	2.01	67.5	
Dibutyl ether	17.85	1.96	140.9	
Diamyl ether	20.51	1.79	170–200	
Dichlorethyl ether	35.08	1.19	178.5	
Diethylene oxide (dioxan)	26.02	1.52	101.5	
<i>Esters</i>				
Methylacetate (Tech.)	19.55	1.84	52–58	
Methylacetate (C.P.)	24.12	1.62	55–58	
Ethyl formate	12.93	2.32	54.3	
Ethyl acetate (Tech.)	19.17	1.87	70–80	
Ethyl acetate (C.P.)	74.00	0.34	77.4	
Butyl acetate	23.00	1.66	100–135	
Amyl acetate	17.21	2.00	125–155	
Octyl acetate	21.01	1.77	199.3	
Methyl amyl acetate	22.06	1.71	145.9	
2-Ethyl butyl acetate	36.63	1.14	162.4	
<i>Ketones</i>				
Acetone (C.P.)	23.12	1.66	56.1	
Methyl acetone (Tech.)	16.90	2.01	50–67	
Diacetone	17.54	1.97	130–180	
Methyl amyl ketone	77.00	0.30	147–154	
Hexone	27.55	1.46	113–119	
<i>Furans</i>				
Furfural			158–162	Too dark to read. Solvent is highly colored. Solvent darkens on standing.
Furfuryl alcohol			165–168	
Tetrahydrofurfuryl alcohol	64.9 in 2 cm. cell	2.45	175–180	

which indicate that maximum extraction is obtained with the tertiary alcohols, decreasing through the secondary and primary alcohols, hydrocarbons, ethers and oxides, esters, halogen derivatives and ketones to a minimum in the aldehydes.

These results were examined in the light of the criteria previously outlined and twelve solvents selected which appeared to fulfil at least certain of these requirements. Further extracts were prepared and spectral distribution curves determined. The results of these tests, together with other information pertinent to selection, are summarized in Table II. On the basis of these data, only *n*-butyl alcohol, methyl-amyl alcohol, dimethylethyl-carbinol, dibutyl ether and ethyl and amyl acetates appeared to warrant consideration. Dimethylethyl carbinol can be ruled out on the score of cost, and dibutyl ether on the

basis of risk of explosion during recovery due to the presence of peroxides. The esters, as commercially available, were found to be variable in composition. This leaves only *n*-butyl and methyl amyl alcohols for final consideration. There appears to be very little to choose between these two solvents; methyl amyl alcohol, however, is slightly more expensive, and the commercial product has a somewhat wider boiling range than *n*-butyl alcohol, indicating a rather lower degree of

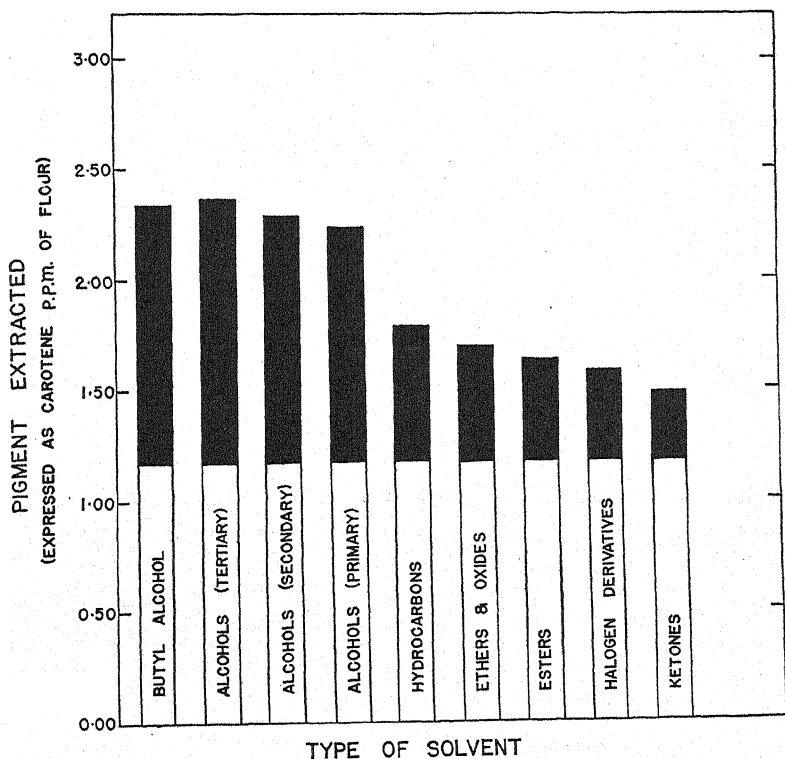


Figure 1. Histogram for means of solvents.

purity. For these reasons, *n*-butyl alcohol was selected and a more extensive series of tests initiated.

Studies with *n*-Butyl Alcohol

Preliminary studies indicated that this solvent showed a tendency to yield slightly turbid extracts which could not be satisfactorily clarified by centrifuging. This difficulty was overcome by saturating the alcohol with water, of which it dissolves about 19.5% of its weight at room temperature. It was ascertained that this treatment does

TABLE II
SUMMARY OF RESULTS OBTAINED WITH TWELVE SELECTED SOLVENTS

Solvent	Trans- mit- tancy (10 cm. cell)	Equi- valent caro- tene	Boiling point	Approx- imate cost	Spectral characteristics of the extract and remarks
	%	P.p.m.	°C.	Cents per pound	
<i>n</i> -Butyl alcohol	12.78	2.33	117.9	26	Carotenoid
Octyl alcohol	9.92	2.62	184.6	28	Carotenoid and flavone
Methyl-amyl-alcohol	13.09	2.32	131.4	28	Carotenoid
Dimethylethyl carbinol	10.56	2.54	99.8–103.8	52	Carotenoid
Dibutyl ether	17.85	1.96	140.9	36	Carotenoid
Ethyl formate	12.93	2.32	54.3	35	Carotenoid and flavone
Ethyl acetate (Tech.)	19.17	1.87	70–80	20	Carotenoid
Amyl acetate (Pentanol)	17.21	2.00	125–155	32	Carotenoid
Diacetone	17.54	1.97	130–180	25	Solvent and extract turn yellow on standing
Propylene dichloride	14.52	2.19	95.9	9½	Flavone with some carotenoid
Xylene	12.18	2.38	144	—	Flavone with some carotenoid
Tetrahydrofurfuryl alcohol	64.9 (2 cm. cell)	2.45	175–180	—	Flavone; very slightly caro- tenoid

not affect either the solvent properties or the spectral characteristics of the extracts which are extremely brilliant and free from any trace of turbidity. The water-saturated solvent appears to flocculate the flour to a marked extent. It might be supposed that the addition of such a considerable amount of water to the solvent would result in an increased extraction of the flavone pigments. That this is not the case is illustrated in Figure 2, which shows spectral distribution curves upon extracts obtained with the new and naphtha-alcohol (93 to 7) solvents from flours milled from high and low grade wheats. The general level of the absorption bands for the butyl alcohol extract is practically identical in both cases, whereas the naphtha-alcohol solvent shows a diminished level in the instance of the low grade sample. This "flattening out" of the carotenoid absorption bands is characteristic of the presence of flavone pigments and shows that the naphtha-alcohol solvent extracts a relatively greater amount of these pigments from the low grade material. Advantage was taken of the "indicator" properties of flavone pigments to confirm this point by re-determining the transmittancies of these extracts after the addition of a solution of anhydrous ammonia in absolute alcohol.

In the presence of flavone pigments, this reagent intensifies the colour, thus reducing the transmittancy and increasing the apparent carotene content. Expressed as carotene, this increase was found to

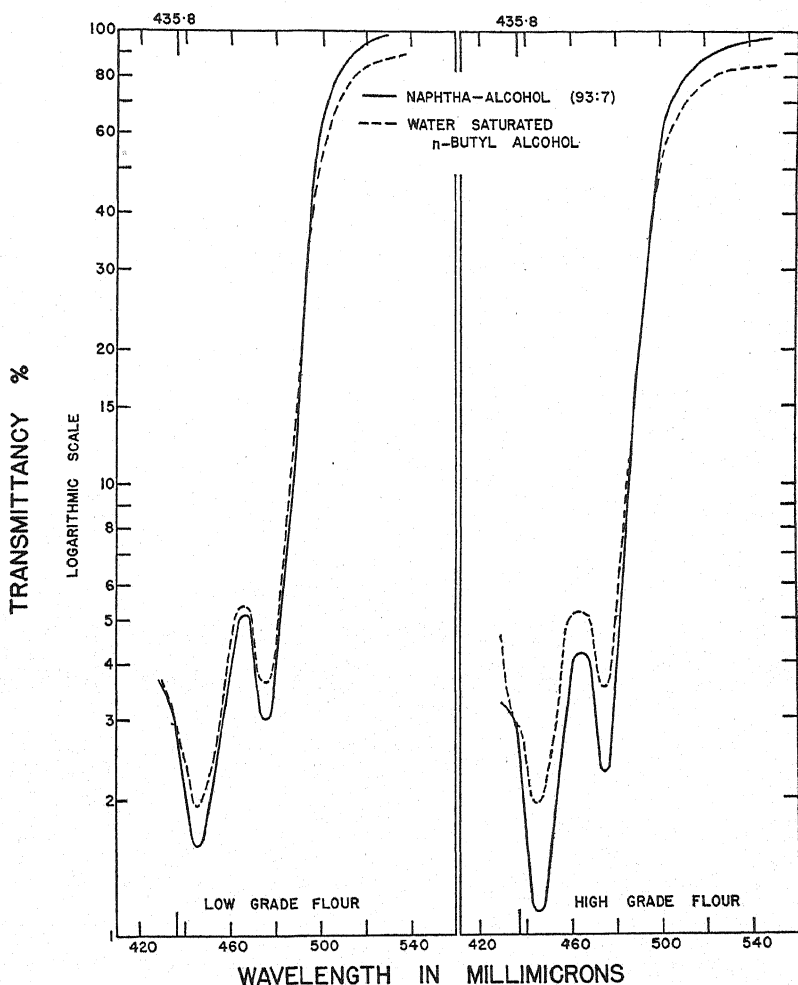


Figure 2. Graph showing spectral distribution curves for N.A. 93:7 and *n*-butyl alcohol, using low and high grade flours respectively.

be 0.44 and 0.49 p.p.m. for the high grade sample in the instance of the naphtha-alcohol and water-saturated *n*-butyl alcohol respectively, whereas for the low grade material the corresponding increases were 0.65 and 0.38 p.p.m.

Specific Transmissive Index (K) for Carotene (and Xanthophyll) in Water-Saturated *n*-Butyl Alcohol, Determined in a 10 Cm. Cell as Wavelength 4358 Å

A determination of this value was essential for conversion of the transmittancy values obtained with the new solvent into equivalent carotene content.

The materials employed consisted of highly purified carotene prepared from carrots and finally recrystallized from carbon disulphide and low boiling petroleum ether (30° to 50° C.). It was dried in vacuo (0.001 mm.) at 50° C. over hard paraffin wax. The xanthophyll was recrystallized from a commercial product.

As a criterion of purity, the specific transmissive index of the carotene was determined in petroleum ether and of the xanthophyll in ethyl ether; the respective values obtained were 1.9145 and 2.094, which are in good agreement with data reported in the literature. Schertz (1923) reported the specific transmissive index of carotene in petroleum ether to be 1.9148 while Ferrari and Bailey (1929) gave a value of 1.9165; for xanthophyll in ethyl ether, Schertz (1925) gave 2.089.

The specific transmissive indices in water-saturated *n*-butyl alcohol were determined by preparing stock solutions containing 5 to 10 mg. per litre of the pigments; dilutions were then made and transmittancies determined. From these data (K) is calculated by the following formula:

$$\text{where } K = \frac{-\log T}{b \cdot c},$$

T = % transmittancy,
 c = concentration of pigment in cg. per litre,
 b = cell length in cm.

The results are detailed in Table III and yield mean values of (K) = 1.6632 for carotene and K = 1.7225 for xanthophyll.

Employing the above specific transmissive index for carotene, a conversion curve or table may be readily constructed in which transmittancy is directly expressed as parts per million of carotene in the sample. Using a 20-gram sample, 100 c.c. of water-saturated normal butyl alcohol, and reading the extract in a 10 cm. cell, the concentration of carotene in parts per million of sample equals 3.006 $[-\log_{10} T]$ and the results which follow were computed from this relation. Recent work on the pigments of wheat and flour (Markley and Bailey, 1935 and 1935a) indicates that they represent a highly complex mixture containing alpha and beta carotenes, xanthophyll and its esters, tricin and similar flavone pigments, together with other unidentified yellow pigments. Under these circumstances the use of a

TABLE III
SPECIFIC TRANSMISSIVE INDICES (K) FOR CAROTENE AND XANTHOPHYLL IN WATER-SATURATED *n*-BUTYL ALCOHOL, DETERMINED IN A 10 CM. CELL AT WAVELENGTH 4358Å

Carotene			
Concentration centigrams per litre	Transmittancy %	$-\log T$	K
.03375	27.50	.56067	1.6612
.04500	17.76	.75056	1.6679
.04950	15.08	.82160	1.6597
.05400	12.63	.89860	1.6640
			Mean 1.6632
Xanthophyll			
.01778	49.50	.30538	1.7175
.03048	29.71	.52710	1.7293
.03810	22.35	.65072	1.7179
.05334	12.02	.92010	1.7250
			Mean 1.7225

conversion table based upon carrot carotene, which is in itself a variable mixture of the alpha and beta isomers, can only be considered as an approximation. However, the spectral characteristics of the extracts secured with the new solvent are essentially carotenoid in character, and until our knowledge of these pigments is extended the employment of some such arbitrary basis of calculation seems essential.

Comparative Results on Hard Red Spring Wheat Flours Employing the Old and New Solvents

It has been indicated that the water-saturated *n*-butyl alcohol extracts more carotenoid pigment from cereal products than naphtha-alcohol. As a considerable volume of data has been accumulated with this latter solvent, it became of interest to determine the relation existing between the two. Accordingly, a series of approximately 150 flours was obtained, and carotene determinations conducted with both solvents. These flours were unbleached and experimentally milled from hard red spring wheats grading No. 1 Hard to No. 6 Special and Nos. 1 and 2 C. W. Garnet.

A summary of the results is given in Table IV, while the relation between the corresponding values on each sample is shown by the scatter diagram in Figure 3. From the latter it will be noted that the difference between the carotene results for the two solvents increases with increasing pigment content, which results in a slightly greater differentiation between samples for the *n*-butyl alcohol solvent. Comparisons between the old and new solvents are being made on ground wheats, durum semolinas and alimentary pastes. The correlation of 0.9325 is quite high and permits of converting carotene

TABLE IV
COMPARISON OF FLOUR CAROTENE VALUES USING NAPHTHA ALCOHOL (93 : 7) AND
WATER-SATURATED *n*-BUTYL ALCOHOL AS SOLVENTS

Solvent	Carotene content (13.5% M.B.)			S.D.	C.V.
	Minimum	Maximum	Mean		
	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>		
Naphtha + alcohol	1.21	2.99	1.77	.354	20.00
<i>n</i> -Butyl alcohol	1.75	3.85	2.33	.470	20.17
	$r_{xy} = .9325$		5% pt. = .195		
	$b_{yx} = 1.2377$				
Regression equation					Standard error of prediction
Carotene, p.p.m. (butyl-alcohol solvent) = $0.14 + 1.2377$ carotene, p.p.m. (naphtha-alcohol solvent)					<i>P.p.m.</i> .170

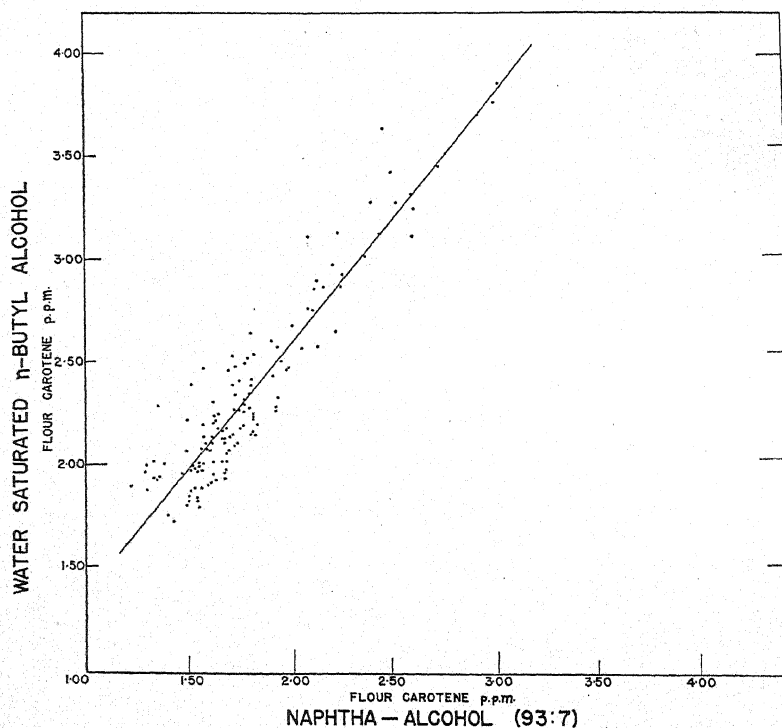


Figure 3. Comparison of results between naphtha-alcohol (93 : 7) and water-saturated *n*-butyl alcohol when applied to 140 hard red spring wheat flours.

results obtained by the naphtha-alcohol to those for the new solvent with a standard error of prediction of 0.170 p.p.m.

Effect of Moisture Content upon the Quantity of Pigment Extracted by Various Solvents

One of the major criticisms of the naphtha-alcohol solvent is its sensitiveness to moisture, either atmospheric or in the sample, and when dealing with flours of rather high moisture content, separation into two phases has frequently been noted. The employment of a water-saturated solvent should tend to minimize such difficulties, and this point was accordingly investigated.

Samples of ground (0.5 mm. Wiley) hard red spring wheat, durum wheat, durum semolina and hard red spring wheat flour were brought to a series of moisture levels by exposure to air of 95% relative humidity for varying lengths of time and pigment determinations conducted using naphtha-alcohol, water-saturated *n*-butyl and methyl-amyl alcohols as solvents. Methyl-amyl alcohol was included because it dissolves considerably less water than *n*-butyl alcohol. The results are summarized in Table V and indicate that variations in moisture have less influence on the carotene values in the instance of the two alcohols, the advantage being slightly in favour of the butyl.

TABLE V

EFFECT OF MOISTURE CONTENT ON QUANTITY OF PIGMENT EXTRACTED BY VARIOUS SOLVENTS

Sample	Range of variation in carotene extracted (13.5% moisture basis)			
	Moisture range	Naphtha-alcohol (93 : 7)	Water-saturated <i>n</i> -butyl alcohol	Water-saturated methyl-amyl alcohol
	%	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>
Hard red spring wheat	8.1-12.7	0.41	0.33	0.39
Durum wheat	8.7-11.6	0.61	0.15	0.20
Hard red spring wheat flour	11.8-15.0	0.08	0.05	0.02
Durum semolina	11.4-14.6	0.62	0.11	0.20

Uniformity of Composition and Solvent Properties

In dealing with petroleum hydrocarbon solvents, various investigators have stressed the necessity of invariably using a specific product or distillate in order to ensure uniform results, but no data appear to be available on the relative solvent properties of different fractions. Two and one-half litres each of "varnish makers' naphtha" and commercial *n*-butyl alcohol were fractionated in all-glass apparatus, twelve fractions of 200 c.c. each being collected. The naphtha fractions were

converted to 93 : 7 solvent by the addition to each of 15 c.c. of absolute ethyl alcohol, and the butyl alcohol fractions were each saturated with water. Extracts were then prepared, using a single large sample of flour, and the extracted pigment determined. The results are presented graphically in Figure 4. The naphtha distilled over a range of from 85° C. to 190° C., the corresponding pigment extracted decreasing steadily from 2.01 p.p.m. to 1.64 p.p.m. The butyl alcohol distillation range was 114.8° C. to 118.2° C. and the corresponding carotene contents varied from 2.27 p.p.m. to 2.30 p.p.m., a total variation of only 0.03 p.p.m., which is practically within the error of the determination. This brief study clearly indicates the undesirability of non-homogeneous solvents and confirms the purity and uniform solvent properties of the commercial butyl alcohol employed in these studies.

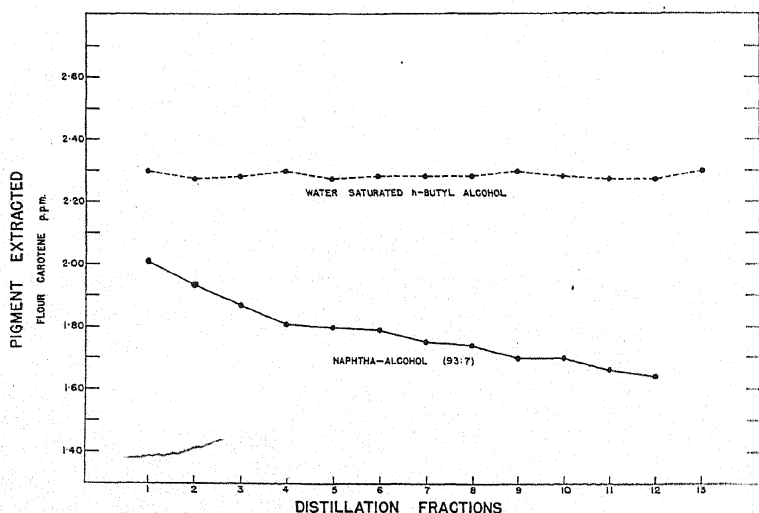


Figure 4. Effect of fractionation of solvent upon the amount of pigment extracted by naphtha-alcohol (93 : 7) and water-saturated *n*-butyl alcohol.

Recovery of Used Solvent

The presence of water in the used butyl alcohol solvent offers no obstacle to recovery by simple distillation. Although the pure alcohol dissolves approximately 19.5% of its weight of water at room temperature, it forms a constant boiling mixture, boiling at 92° C. and containing 37% of water, which mixture on cooling separates into two phases. As the elimination of water from the mixture in the distilling flask proceeds, the temperature rises to the boiling point of the anhydrous alcohol (117.9° C.) and remains at this point until distillation is complete. It is then simply necessary to add a small excess of

water to the distillate, shake thoroughly in order to ensure complete saturation, and allow to stand until clear.

Discussion

Considered in the light of the criteria established for a satisfactory solvent, water-saturated *n*-butyl alcohol possesses high solvent power for the carotenoids and relatively low solvent power for the flavone pigments of flour. The extracts obtained are easily centrifuged or clarified, and are extremely brilliant and free from turbidity; it flocculates the flour to a marked extent. The purity of the commercial product is high, and the various fractions obtained upon distillation possess practically identical solvent properties. The boiling point is moderate (118° C.), the flash point relatively high (43.9° C.), the odor not pronounced, and the toxicity low. As prepared for use by saturation with water, this solvent is unaffected by atmospheric moisture and minimizes the disturbing effect of sample moisture content. It is readily recovered for re-use by simple distillation and in view of this ease of recovery is comparatively inexpensive.

Summary

The requirements of an ideal solvent for wheat and flour pigments are detailed, the deficiencies of petroleum hydrocarbons and admixtures for this purpose are pointed out and the necessity for a more suitable and chemically homogeneous solvent indicated.

Sixty commercially available solvents were submitted to a preliminary survey and a general relation found between efficiency of extraction and chemical constitution. In decreasing order this is: tertiary alcohols, secondary alcohols, primary alcohols, hydrocarbons, ethers and oxides, esters, halogen derivatives, ketones and aldehydes. Twelve of these were subjected to a more detailed study and *n*-butyl alcohol finally selected as the most satisfactory.

This solvent is employed saturated with water and yields brilliant extracts which are essentially carotenoid in character. The specific transmissive indices of carrot carotene and xanthophyll in this solvent are 1.6632 and 1.7225 respectively, determined in a 10 cm. cell at 4358 Å.

Carotene values for 140 hard red spring wheat flours determined with the water-saturated *n*-butyl alcohol and naphtha-alcohol respectively indicate that the former extracts more pigment, particularly in the instance of highly pigmented flours; the correlation found between corresponding values was 0.9325.

The use of water-saturated *n*-butyl alcohol minimizes the disturbing effect of variations in sample moisture which are quite pronounced with naphtha-alcohol.

The purity of commercial *n*-butyl alcohol is high (boiling range 115° to 118° C.) and the various fractions obtained upon distillation possess practically identical solvent properties, thereby permitting recovery for re-use by simple distillation. On the other hand, naphtha has a very wide boiling range and the various fractions differ widely in solvent power.

N-butyl alcohol fulfils the majority of the requirements set forth for an ideal solvent and is regarded as decidedly preferable to petroleum distillates or their admixtures with ethyl alcohol.

Literature Cited

- American Association of Cereal Chemists
1935 Cereal Laboratory Methods. Third Ed. Published by American Association of Cereal Chemists.
- Coleman, D. A., and Christie, Alfred
1926 A rapid method for determining the gasoline color value of flour and wheat. *Cereal Chem.* 3: 84-89.
- Ferrari, C. G.
1933 Spectrophotometric determination of the carotenoid pigment content of wheat flour. *Cereal Chem.* 10: 277-286.
- , and Bailey, C. H.
1929 The carotenoid pigments of flour. *Cereal Chem.* 6: 218-240.
1929a The determination of carotene in flour. *Cereal Chem.* 6: 347-371.
- Geddes, W. F., Binnington, D. S., and Whiteside, A. G. O.
1934 A simplified method for the determination of carotene in flour extracts. *Cereal Chem.* 11: 1-24.
- Johannson, H.
1935 A study of the carotenoid pigments of wheat and five types of rust spores. Master's Thesis, University of Manitoba, Winnipeg, Manitoba.
- Kent-Jones, D. W.
1927 Modern Cereal Chemistry, Second Ed., p. 378. Northern Publishing Company, Liverpool, England.
- Markley, M. C., and Bailey, C. H.
1935 The nature of the pigments of the gasoline extract of wheat. *Cereal Chem.* 12: 33-39.
1935a The pigments of the dilute alcohol or acetone extract of whole wheat meal. *Cereal Chem.* 12: 40-48.
- Schertz, F. M.
1923 The quantitative determination of carotene by means of the spectrophotometer and colorimeter. *J. Agr. Research* 26: 383-440.
1925 The quantitative determination of xanthophyll by means of the spectrophotometer and the colorimeter. *J. Agr. Research* 30: 253-261.
- Simpson, A. G.
1935 A simple method for determining the "yellowness" and "grade" of wheat flours. *Cereal Chem.* 12: 569-574.
- Whiteside, A. G. O.
1931 A study of the carotenoid pigments of wheat and flour with special reference to wheat varieties. Dom. Dept. Agr. Bul. No. 154 (new series).
- Winton, A. L.
1911 Color of flour and a method for the determination of "gasoline color value." U. S. D. A. Bur. Chem. Bul. No. 137.

BARLEY AND MALT STUDIES. IV. EXPERIMENTAL MALTING OF BARLEYS GROWN IN 1936 AND SUMMARY DATA FOR THREE YEARS 1934-1936¹

JAMES G. DICKSON, ALLAN D. DICKSON, H. L. SHANDS,
and B. A. BURKHART²

University of Wisconsin and Division of Cereal Crops and Diseases, B. P. I.,
U. S. D. A., Madison, Wisconsin

(Read at the Annual Meeting, May 1937)

Introduction

The regional investigations on the malting quality of barleys during three crop years have been completed. The investigations on the 1934 and 1935 crops were reported previously in annual summaries (Dickson, Shands, Dickson, and Burkhart, 1935, 1937): Tabular data were not presented in the two earlier reports primarily because it seemed advisable to accumulate three years' results and to test more adequately the methods of malting and of evaluating malts before the data were presented in detailed form.

The present paper, therefore, includes a discussion of the experimental malting of the barleys grown in 1936, and the three years' data for the five varieties grown at the cooperating agricultural experiment stations. The statistical analysis of the three-year data is presented in abbreviated form to show the relationship of variety, location and season in influencing malting quality. The analysis has been used to determine the response of varieties to locations, seasons, and years.

The three years' data, the writers believe, are not enough to warrant conclusive statements regarding malting quality or methods. Seasonal conditions during the three years have been shown to play such an important rôle in the determination of quality that additional investigations are necessary before final conclusions are drawn. Some of the stations have not submitted samples in all of the three years, thus making the data for those particular stations of less value for the

¹ Based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station. The cooperative investigations include the agricultural experiment stations of California, Colorado, Illinois, Iowa, Michigan, Minnesota, Montana, Nebraska, North Dakota, Ohio, South Dakota and Wisconsin, where the uniform barley varietal series are grown each year. The Federal W.P.A. has contributed to the research program during the past year through a grant under the University of Wisconsin W.P.A. Project 425. The United States Maltsters Association has cooperated through an industrial fellowship grant to the University of Wisconsin.

² Recognition is given G. A. Wiebe, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, for assistance in the analysis of the data and the preparation of the manuscript.

regional study of malting quality. The writers feel, however, that the results should be presented in some detail to serve as a report of the investigations in progress.

The investigations on the development of methods for the evaluation of malting quality have been continued. The study of the malting procedure on an experimental scale and the comparison of the malts produced with those made by the commercial houses have required considerable time. The investigations on the physiology of malting have been continued to determine the relative importance of moisture, temperature, and time in processing different lots of barley. The results of these physiological investigations have been useful in the interpretation of results obtained in the routine procedure employed in the comparison of barleys in the regional study.

The data presented in this paper were obtained from barleys grown at a number of cooperating experimental stations and a great deal of credit is due the agronomists at these stations. It is by such co-operation that a study of this kind is made possible.

Varieties Used

Five varieties of barley were used in this study. A brief description of each follows:

Oderbrucker (Wisconsin Pedigree 5-1) is a six-rowed, rough-awned variety with white kernels. Oderbrucker was selected at the Wisconsin Agricultural Experiment Station from stock obtained from the Ontario Agricultural College at Guelph. The original importation was from Germany. The Wisconsin Oderbrucker was distributed in 1908.

Wisconsin Barbless (Wisconsin Pedigree 38) is a six-rowed, smooth-awned white-kerneled variety. It is a variety of hybrid origin selected from the cross Oderbrucker $\times$ Lion at the Wisconsin Agricultural Experiment Station. This variety was released to the farmers in 1929-1930.

Velvet (Minnesota No. 447) is a six-rowed, smooth-awned, white-kerneled variety. Velvet is a selection from a cross of a smooth-awned segregate of Manchuria $\times$ Lion with Luth (a Manchuria selection). It was produced by the cooperative work of the Minnesota Agricultural Experiment Station and the United States Department of Agriculture. It was released in 1926.

Manchuria (North Dakota 2121) is a six-rowed, rough-awned variety having both blue and white kernels. It was selected from an old Manchuria variety at the North Dakota Agricultural Experiment Station in 1901. It was distributed to farmers in 1920.

Trebi (C. I. No. 936) is a six-rowed, rough-awned variety and has large blue kernels. It is a selection from an imported lot of seed obtained by the United States Department of Agriculture from the south side of the Black Sea in 1905. The selection was made in 1909 by H. V. Harlan, of the United States Department of Agriculture, at the Minnesota Agricultural Experiment Station. It was released for commercial production in 1918.

Study of Malting Controls

The checking of methods and procedures of malting in the comparative study of a large number of barleys has been accomplished in part by the use of "malting controls." For this purpose relatively large lots of barley were obtained each year from the bulk grain produced in the increase fields on the University Farms at Madison. These lots were cleaned, thoroughly mixed and stored, and were used as controls in each of the malting series and for special studies on methods. The Wisconsin Barbless was used in 1934 and 1935. Two controls were used in 1936, Wisconsin Barbless and Oderbrucker.

The malting controls were included with each group of barleys malted and analyzed. In addition the controls from the previous years were used in one or two of the first series of barleys malted the following year in order to have a direct comparison between the barleys of the successive seasons and to check methods of malting by comparing with the previous year's results. The data obtained from these malting controls have been used in determining the variability between the five to ten different malting runs made each year in the malting chamber. The summary data on variability of the malting controls for the past three years and standard deviations are presented in Table I.

The data show considerable range in variability for the different determinations. The maximum variation in the moisture content reached in the steep in 1934-1935 was 0.9% above and below the mean. The moisture content reached in the steep in the 1935-1936 Wisconsin Barbless controls varied from 1.3% above to 1.1% below the mean moisture content with the same steep time. The moisture content reached in the steep for Wisconsin Barbless in the 1936-1937 runs was higher (mean moisture content 48.1%) and varied from less than 0.4% below in one malting to 0.8% above. The maximum variation in the moisture content of the steeped barley in the Oderbrucker control was 1.1% above and 0.5% below the mean. The moisture content of the barley at the end of the six-day germination period was more variable than after steeping. The maximum variation in moisture content above and below the mean was as follows: in 1934, 1.6% above, 0.5%

TABLE I

VARIABILITY OF MALTING CONTROLS USED IN EACH MALTING SERIES IN THE LARGE UNIT. SUMMARY OF FIVE SERIES WITH ONE VARIETY IN 1934-1935, EIGHT SERIES WITH ONE VARIETY IN 1935-1936, AND SEVEN SERIES EACH FOR TWO VARIETIES IN 1936-1937¹

Barley variety and year	Calculations	Moisture content reached in steep	Moisture content at end of germination	Moisture content of dried malt	Recovery of malt from barley dry basis	Kernel weight of malt dry basis	Extract dry basis	Total nitrogen in malt	Soluble nitrogen in wort	Dia-static power
		%	%	%	%	Mg.	%	%	%	°L.
Wisconsin Barbless Barley sample No. 46, 1934-1935	Mean	45.5	49.3	3.0	84.5	28.5	70.9	2.43	0.681	72.0
	Standard deviation	.663	.870	.870	1.035	.424	.794	.041	.031	13.89
	5% point	1.9	2.5	2.5	2.9	1.2	2.2	0.12	0.09	39.3
5 series	Coefficient of variability	1.458	1.766	28.818	1.225	1.489	1.120	1.706	4.599	19.296
Wisconsin Barbless Barley sample No. 336, 1935-1936	Mean	45.4	46.5	5.2	87.1	26.94	74.9	2.10	0.646	137.1
	Standard deviation	.889	.926	.621	2.009	.672	.441	.037	.017	10.37
	5% point	2.0	2.1	1.4	4.6	1.5	1.0	0.07	0.04	23.9
8 series	Coefficient of variability	1.960	1.990	12.000	2.310	2.490	0.588	1.765	2.576	7.56
Wisconsin Barbless Barley sample No. 466, 1936-1937	Mean	48.1	45.6	4.5	91.0	22.76	67.8	2.19	.593	97.3
	Standard deviation	.441	.689	.619	1.249	.316	.138	.037	.017	10.20
	5% point	1.0	1.6	1.5	2.9	0.7	0.3	0.09	0.04	24.1
7 series	Coefficient of variability	.910	1.510	13.720	1.370	1.390	.200	1.715	2.920	10.480
Oderbrucker Barley sample No. 464, 1936-1937	Mean	46.3	45.4	4.8	89.5	23.67	73.7	1.99	0.674	146.6
	Standard deviation	.297	.573	.570	.772	.706	.368	.120	.012	18.57
	5% point	0.7	1.3	1.3	1.8	1.7	0.9	0.28	0.03	43.9
7 series	Coefficient of variability	.640	1.260	11.850	.860	2.980	.499	6.027	1.720	12.670

¹ Barleys were all grown for six days in malting chamber.

below; in 1935, 1.3% above, 1.2% below; and in 1936, 1.1% above, 0.7% below for Wisconsin Barbless, and 3.8% above and 2.3% below the mean for Oderbrucker. This was due in part to the slight variations in relative humidity within the chamber, especially during the last day of germination, and represents more variability than actually existed during the germination period because water was added to bring the samples to a given weight each day. The moisture content of the dried malt was rather variable in terms of the coefficient of variability. The actual variation, however, was less than 1% above and below the mean except in the malting series of the first year, which ranged from 2.1 to 4.0% moisture content in the dried malt. The mean recovery of malt from barley was significantly lower for Wisconsin Barbless in 1934-1935 than in the other two series. The somewhat higher moisture content used during the germination period and perhaps the use of less accurate methods of determining recovery were partly responsible for this lower recovery. The variability in kernel weight due to the combined influence of malting and determination was greater in three out of the four series than the limits suggested

by Shands (1937) for determinations alone. The variability in kernel weight in the malting controls, however, was not correlated with moisture content at the end of the germination period, which suggests that the range of variation in kernel weight in these series was not greatly influenced by malting conditions. Moisture studies have shown that there is a high correlation at the end of the germination period between moisture content and kernel weight. The total nitrogen was highly variable, especially in the Oderbrucker controls. Experiments to determine the variability in analytical methods, not reported in the present paper, suggest that most of this error has been in sampling the ground material for nitrogen determinations. The accuracy has been improved recently by the use of the Wiley Mill to grind the malts for moisture and nitrogen determinations. Diastatic power has shown a variability higher than any of the other determinations except moisture content of the dried malt. Inasmuch as diastatic power is greatly influenced by drying conditions and the moisture content to which the malts are dried, there is evidently a direct relationship between the variability in moisture content and diastatic power. The variability in extract yield was relatively low in all cases, with an average standard deviation of 0.437% or approximately twice that for the determination of extract alone.

The variability between malting controls, as presented in summary form in Table I, includes all sources of variation between the different runs of a given year. The variation within individual runs has been much less. The methods in general, however, have been sufficiently accurate to evaluate differences between varieties, stations and seasons.

The variation between different malting runs in the malting chamber, while accurate enough to show differences between varieties, stations and years, needs further study especially with relation to moisture control in the chamber and to drying to a more uniform moisture content. Changes of 2 to 3% in moisture content during the germination process are reflected in malt recovery from the barley, in soluble nitrogen in the wort and in diastatic power of the malts. Improvements in moisture control in the malting chamber by means of a humidistatic control of the water spray in the chamber and some means of stopping drying of the different samples at more nearly the same moisture content will do much to reduce the variations now present in the malting procedure.

Comparison of Five Varieties of Barley Grown in Uniform Regional Series in 1936

Four varieties of barley, Oderbrucker, Wisconsin Barbless, Velvet, and Manchuria, were grown at eleven experiment stations in 1936.

Trebi was grown at ten stations. The seasonal conditions at most of the stations in the upper Mississippi Valley were very unfavorable due to the hot, dry weather during the heading and ripening period. The unfavorable weather conditions placed the earlier maturing varieties at a comparative advantage in yield, plumpness of kernel and malting quality. In general, however, the varieties remained in essentially the same relative order for the major malting factors at the different stations and in the average for all stations as in the two previous years. The data for the barleys grown in 1936 are presented in comparison with the results for the two previous years in Tables II to XXII and in summary form for the five varieties in Table XXIII. Location where grown appears to be the greatest factor influencing yield, with variety and year less important.

The influence of the hot, dry season and the period of maturity of the varieties upon quality is well illustrated in the five varieties grown at the widely separated stations. Yields of all varieties were reduced in 1936. The test weight of Wisconsin Barbless barley was below that of the two previous years. The relative differences between 1935 and 1936 in yield, kernel weight and extract of Wisconsin Barbless, in contrast to Manchuria and Velvet, show the different reaction of late and early maturing varieties. (See Tables II, III, V and XV, and

TABLE II
AVERAGE YIELD PER ACRE OF THE FIVE VARIETIES OF BARLEY GROWN IN THE
REGIONAL SERIES IN 1934, 1935 AND 1936

Variety	Year	Location where barley was grown													Average ^a
		East Lan- sing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmetts- burg, Iowa, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
		Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.
Oder- brucker	1934	27.5	—	—	10.0	13.8	19.8	—	11.7	21.5	—	46.8	59.7	—	26.3
	1935	25.6	39.5	—	43.0	40.4	35.3	21.1	15.3	25.0	17.7	58.3	32.6	—	32.2
	1936	31.8	42.9	30.8	16.1	36.5	39.5	28.5	11.9	—	—	54.1	56.7	20.0	33.5
Wisconsin Barbless	1934	35.9	—	—	10.7	22.1	30.5	—	25.2	31.1	—	80.5	—	—	33.7
	1935	45.8	45.7	—	57.0	72.3	43.6	38.7	27.8	48.7	28.7	82.8	65.4	—	50.6
	1936	43.0	45.9	35.5	18.9	45.0	43.5	32.9	13.1	—	—	70.4	74.4	21.0	40.3
Velvet	1934	29.6	—	—	15.3	18.5	29.9	—	22.3	30.0	—	73.9	85.2	—	38.1
	1935	37.5	41.6	—	47.0	56.5	30.5	30.4	21.7	42.3	18.4	82.4	45.4	—	41.2
	1936	36.7	45.7	30.2	15.0	39.4	44.4	27.1	16.9	—	—	72.0	68.7	14.0	37.3
Manchuria	1934	21.6	—	—	14.3	15.8	31.4	—	18.7	29.6	—	74.9	59.4	—	33.2
	1935	24.2	40.8	—	41.5	53.5	27.1	17.3	13.6	46.4	13.9	68.1	20.3	—	34.1
	1936	25.7	36.7	29.1	6.8	38.0	41.8	24.6	11.9	—	—	57.2	56.7	23.0	32.0
Trebi	1934	32.7	—	—	18.6	25.3	39.6	—	44.6	38.0	—	126.8	73.6	—	49.9
	1935	29.0	53.0	—	46.8	66.9	39.2	27.4	29.9	61.9	17.7	92.9	32.8	—	45.2
	1936	34.3	65.7	36.5	19.0	55.9	63.5	42.5	24.8	—	—	80.5	78.9	—	50.2

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

² Averages of all stations reporting for each year, therefore not comparable between years. See Table XXIII for comparable averages for six stations.

TABLE III
BUSHEL WEIGHT OF BARLEY IN POUNDS, DRY BASIS, OF THE FIVE VARIETIES OF
BARLEY GROWN IN THE REGIONAL SERIES IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ³
		Columbus, Ohio	East Lan- sing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmets- burg, ¹ Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	
	1935	39.3	—	—	—	36.7	42.4	40.4	—	41.6	42.4	—	43.8	41.9	—	41.1
	1936	34.7	40.6	41.9	—	41.6	43.1	—	34.8	26.1	26.9	—	45.6	45.1	46.4	38.9
Wisconsin Barbless	1934	—	40.3	42.5	44.6	38.5	41.1	41.6	40.5	32.1	—	—	43.8	42.0	44.9	41.1
	1935	36.0	40.1	40.2	—	42.0	43.1	—	38.6	30.7	34.4	39.9	45.9	46.1	46.5	40.3
	1936	—	35.6	39.2	40.9	35.8	40.9	40.6	39.5	30.5	—	—	43.6	41.0	45.3	39.4
Velvet	1934	—	40.0	—	—	36.1	41.4	39.6	—	43.5	41.4	—	44.5	42.2	—	41.1
	1935	20.6	39.5	41.4	—	41.1	44.2	—	39.2	30.9	33.2	39.6	44.9	46.3	—	38.3
	1936	—	38.1	42.0	45.1	37.5	42.0	42.4	42.8	35.1	—	—	43.4	41.4	45.2	41.4
Manchuria	1934	—	40.2	—	—	36.0	41.4	37.3	—	43.8	39.4	—	44.4	39.8	—	40.3
	1935	35.7	38.4	39.6	—	40.6	43.8	—	35.8	30.0	31.3	36.6	46.3	46.1	46.6	39.2
	1936	—	35.9	42.2	45.0	37.2	41.1 ²	43.4	36.2	33.6	—	—	43.9	39.8	43.7	40.2
Trebi	1934	—	40.8	—	—	34.6	39.9	37.7	—	40.1	39.6	—	43.5	40.0	—	39.5
	1935	31.1	37.4	40.0	—	39.4	42.4	—	35.4	33.3	34.1	37.0	44.5	44.9	—	38.1
	1936	—	34.8	43.1	43.7	37.2	40.3	41.2	40.0	35.2	—	—	41.5	39.9	—	39.7

¹ Barleys grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.² Manchuria (Minn. 184) was substituted for Manchuria (N. Dak. 2121), which was not furnished from the Waseca station in 1936.³ Averages of all stations reporting for each year, therefore not comparable between years. See Table XXIII for comparable averages for six stations.

TABLE IV
BUSHEL WEIGHT OF MALT IN POUNDS, 4% MOISTURE BASIS, FOR MALTS FROM THE
FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL
SERIES IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ³
		Columbus, Ohio	East Lan- ang, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmets- burg, ¹ Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oberbrucker	1934	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.
	1935	33.5	35.3	39.1	—	33.9	36.9	—	—	36.5	36.1	—	40.0	36.8	—	36.5
	1936	—	36.3	37.4	38.4	38.9	35.4	—	32.4	24.0	32.2	33.3	40.6	39.8	38.7	34.5
Wisconsin Barbless	1934	—	35.4	—	—	33.9	36.6	—	—	37.1	37.2	—	39.7	—	—	36.6
	1935	34.9	36.2	37.4	—	38.8	37.7	—	35.4	29.6	—	36.0	42.8	40.8	41.3	37.0
	1936	—	33.5	35.1	36.6	31.8	37.5	34.2	33.8	27.2	—	—	41.2	37.6	39.5	35.3
Velvet	1934	—	34.6	—	—	34.6	35.7	—	—	38.3	36.2	—	40.0	37.6	—	36.7
	1935	42.4	36.1	38.2	—	38.2	37.1	—	35.3	28.8	35.2	34.2	40.9	41.2	—	37.0
	1936	—	34.8	36.0	38.1	33.8	35.2	35.0	34.6	30.7	—	—	39.2	36.6	39.1	35.7
Manchuria	1934	—	35.5	—	—	34.3	35.5	—	—	36.4	40.3	—	39.3	35.4	—	36.7
	1935	32.0	34.2	34.7	—	37.2	35.9	—	32.8	26.3	29.4	33.3	40.2	40.7	40.6	34.8
	1936	—	33.0	35.3	37.4	32.1	34.3 ²	34.3	33.5	28.4	—	—	37.9	36.1	35.4	34.4
Trebi	1934	—	34.9	—	—	33.2	34.1	—	—	35.1	35.7	—	39.4	36.3	—	35.5
	1935	31.8	35.6	36.3	—	36.6	41.2	—	34.4	30.2	—	31.2	36.3	39.8	—	35.3
	1936	—	33.0	37.2	38.3	32.8	34.2	35.3	34.7	27.7	—	—	38.1	35.9	—	34.7

¹ Barleys grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121) at Waseca in 1936 series.³ Averages for all stations reporting for each year, therefore not comparable between years. See Table XXIII for comparable averages from six stations.

summary Table XXIII.) There is likewise a suggestion as in the previous years that the Manchuria and Velvet varieties can withstand dry conditions better than the Oderbrucker and Wisconsin Barbless barleys without impairing malting quality. The contrast between the barleys grown at the upper Mississippi Valley stations and those grown at the stations in the irrigated Intermountain area and California also shows the influence of seasonal conditions upon malting quality (Tables III to XX). The 1936 averages for the varieties

TABLE V[✓]

KERNEL WEIGHT OF BARLEY IN MILLIGRAMS, DRY BASIS, EXCEPT 1934 DATA WHICH ARE ON AIR-DRY BASIS. FIVE VARIETIES OF BARLEY GROWN IN REGIONAL SERIES IN 1934, 1935, AND 1936. CALCULATED ON THE BASIS OF WEIGHT OF 400 KERNELS

Variety	Year	Location where barley was grown															Average ⁴
		Columbus, Ohio	East Lansing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmetsburg, ² Ia.	Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934 ¹	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>
	1935	25.7	33.2	29.1	—	33.3	30.7	29.9	—	34.0	29.6	—	35.4	28.5	—	31.8 ¹	31.8 ¹
	1936	—	29.6	26.7	29.1	24.8	22.5	22.9	21.7 ²	12.4	14.8	—	21.0	32.6	32.8	34.4	25.9
Wisconsin Barbless	1934 ¹	—	34.0	—	—	35.9	29.2	30.3	—	35.9	30.4	—	37.2	—	—	—	33.3
	1935	26.0	29.5	29.7	—	31.7	28.8	—	24.0	15.8	22.3	24.9	34.4	31.7	38.5	28.1	33.3
	1936	—	28.0	26.7	25.2	22.8	25.6	25.7	24.5	18.8	—	—	35.7	27.4	33.7	26.7	26.7
Velvet	1934 ¹	—	31.5	—	—	32.3	27.3	29.0	—	33.9	29.5	—	36.1	26.9	—	—	30.8
	1935	21.8	26.1	29.3	—	28.6	25.0	—	21.3	14.7	18.3	21.9	31.0	33.1	—	24.6	30.8
	1936	—	27.5	25.8	28.8	24.1	24.0	22.1	24.3	19.7	—	—	31.1	27.8	28.4	25.8	24.6
Manchuria	1934 ¹	—	31.1	—	—	31.2	26.8	26.1	—	31.3	27.4	—	34.7	23.8	—	—	29.0
	1935	21.7	25.6	25.8	—	29.1	25.1	—	19.1	13.9	19.1	19.6	31.7	28.3	32.6	24.3	29.0
	1936	—	25.3	25.4	26.7	23.8	22.8 ³	23.4	21.7	19.9	—	—	30.1	24.3	28.2	24.7	24.7
Trebti	1934 ¹	—	42.8	—	—	41.6	37.7	40.2	—	40.9	40.6	—	42.9	36.1	—	—	40.4
	1935	28.8	34.8	35.4	—	38.1	34.5	—	28.3	23.1	27.1	27.2	40.5	36.5	—	32.2	40.4
	1936	—	30.1	37.5	39.1	32.1	30.4	33.0	31.6	27.4	—	—	39.2	32.1	—	33.3	33.3

¹ Air-dry basis for 1934, all other weights on dry basis. The averages for kernel weights in 1934 calculated on the dry basis by using 12% moisture for the barley are as follows: Oderbrucker 28.0, Wisconsin Barbless 29.3, Velvet 27.1, Manchuria 25.5, Trebti 35.5 mg., respectively.

² Barleys grown at Ames in 1936, and at Emmetsburg, Iowa, in 1935.

³ Manchuria (Minn. 184) data given instead of Manchuria (N. Dak. 2121), as the latter variety was not grown in 1936.

⁴ Averages for all stations; see Table XXIII for comparable averages.

grown at all stations are not greatly different from the general averages in the 1935 season. (See averages given in Tables II to XXII.) A study of the data for the barleys from the stations in the Mississippi Valley area in 1936 and the averages for the six stations supplying samples for each of the three years shows a marked inferiority in the quality of those barleys as compared with the barleys produced in 1935 (Table XXIII). The barleys from these stations more nearly reflect the general low quality of the 1936 crop of barley than do the averages from all of the stations. The survey of the individual factors used in

evaluating the barleys and malts shows in more detail the seasonal and regional effects upon quality.

The yields of the five varieties of barley varied widely at the different stations. The average yield of the varieties at the six comparable stations for the three years was lower than in 1935 (Table XXIII). The average yield of Oderbrucker at the eleven stations reporting was higher in 1936 than the average for all stations in either of the previous seasons. On the same basis of averaging, Velvet and Manchuria produced yields only slightly less than in 1935. The aver-

TABLE VI ✓

KERNEL WEIGHT OF MALT IN MILLIGRAMS, DRY BASIS, FROM BARLEYS GROWN IN REGIONAL SERIES IN 1934, 1935, AND 1936. CALCULATED ON THE BASIS OF 400 KERNELS

Variety	Year	Location where barley was grown														Average ³
		Columbus, Ohio	East Lansing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmettsburg, ¹ Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
Oderbrucker	1934	—	26.1	—	—	26.9	24.1	24.9	—	27.4	23.2	—	28.0	23.2	—	25.6
	1935	21.8	25.0	25.3	—	28.4	21.5	—	17.2 ²	11.2	19.0	18.6	28.4	26.6	28.5	22.6
	1936	—	26.4	24.6	25.7	20.6	20.8	20.7	20.4 ¹	16.1	—	—	26.7	25.0	26.4	23.0
Wisconsin Barbless	1934	—	27.5	—	—	30.4	23.8	25.4	—	20.2	23.5	—	31.5	—	—	26.0
	1935	22.6	25.5	25.0	—	27.9	25.9	—	20.7	14.8	—	22.6	30.0	27.1	32.9	25.0
	1936	—	32.4	23.4	22.9	21.2	20.5	22.2	21.6	16.3	—	—	31.9	24.5	29.5	24.2
Velvet	1934	—	27.0	—	—	25.2	20.4	23.8	—	27.1	22.3	—	29.5	21.8	—	24.6
	1935	18.8	22.1	25.4	—	24.3	22.1	—	16.9	12.8	15.8	18.5	26.8	28.3	—	24.6
	1936	—	23.8	23.5	25.0	20.4	22.1	20.7	22.1	17.4	—	—	29.0	24.3	25.8	23.1
Manchuria	1934	—	25.4	—	—	23.8	20.3	22.2	—	24.1	21.1	—	27.0	19.6	—	22.9
	1935	19.7	22.2	21.5	—	25.5	21.7	—	17.0	12.5	15.9	17.8	27.7	24.3	28.3	21.2
	1936	—	23.2	22.3	23.2	21.3 ²	19.6	21.2	20.2	13.8	—	—	25.7	21.7	25.3	21.6
Trebi	1934	—	34.9	—	—	28.7	28.9	35.2	—	33.4	31.5	—	38.7	29.8	—	32.6
	1935	24.4	30.7	30.4	—	33.0	36.9	—	25.0	19.7	—	23.1	31.7	32.6	—	28.7
	1936	—	28.5	33.4	34.5	29.4	27.3	28.7	29.2	19.6	—	—	36.2	28.8	—	29.6

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121), as latter not grown at Waseca with series in 1936.

³ Averages for all stations; see Table XXIII for comparable averages.

age production of Wisconsin Barbless was 10 bushels below 1935. The average yield for Trebi was higher than in either of the previous years. The contrast between yields at the different stations shows clearly the relative behavior of the varieties as well as seasonal conditions at the different stations. The yield data for the three years are given in Table II. The average yields for the six stations that were used in the analysis of variance are given in Table XXIII.

The bushel weight of the 1936 barleys was in general rather low for all varieties grown at most locations in the upper Mississippi Valley area. The average bushel weight of barley and malt from the six

TABLE VII
SKINNED AND BROKEN KERNELS FOR THE FIVE VARIETIES OF BARLEY GROWN IN
THE REGIONAL SERIES IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ³
		Columbus, Ohio	East Lan- sing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmets- burg, ¹ Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
	1935	0.7	7.9	2.3	—	7.8	14.5	5.1	—	5.9	3.6	—	0.9	6.0	—	6.5
	1936	—	1.7	2.4	7.4	2.1	11.7	1.4	3.2	1.4	0.5	2.4	5.2	—	6.8	3.1
		—	—	—	—	—	—	—	3.3	5.7	—	—	4.8	—	15.1	5.5
Wisconsin Barbless	1934	—	9.3	—	—	5.3	3.7	3.9	—	9.0	4.9	—	1.2	—	—	5.4
	1935	2.7	2.2	3.4	—	2.3	7.0	—	5.4	0.9	3.2	5.7	10.4	24.2	11.4	6.6
	1936	—	0.3	1.6	5.8	1.5	2.4	0.7	4.5	4.9	—	—	7.4	5.7	22.7	5.2
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Velvet	1934	—	9.2	—	—	5.4	7.3	1.1	—	8.5	5.3	—	1.0	5.1	—	5.4
	1935	2.9	1.4	0.8	—	2.7	10.1	—	4.9	1.6	0.6	4.8	8.6	19.3	—	5.2
	1936	—	1.3	1.9	4.8	2.4	9.0	1.0	2.8	3.5	—	—	3.9	12.9	21.7	5.9
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Manchuria	1934	—	15.1	—	—	2.9	6.8	1.7	—	14.5	4.1	—	1.6	4.4	—	6.4
	1935	1.0	2.0	2.1	—	1.7	11.8	—	2.3	2.4	1.0	4.7	6.5	18.1	5.4	4.9
	1936	—	0.6	2.9	6.8	2.2	16.2 ²	3.2	3.2	7.9	—	—	6.4	4.2	15.3	6.3
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Trebi	1934	—	6.2	—	—	2.6	11.9	3.4	—	5.2	3.2	—	1.1	3.8	—	4.7
	1935	1.1	1.0	0.7	—	2.2	7.0	—	4.3	2.6	2.5	8.8	16.2	28.9	—	6.8
	1936	—	4.0	2.3	7.5	6.0	30.0	1.7	8.6	4.6	—	—	4.8	7.7	—	7.7
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Station average		1.7	4.2	2.0	6.5	3.3	10.2	2.3	4.2	5.2	2.9	5.3	5.3	11.0	14.1	—

¹ Barley grown at Emmetsburg in 1935, and at Ames in 1936.

² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121) in Waseca series in 1936.

³ Averages for all stations; see Table XXIII for comparable averages.

TABLE VIII
WEIGHT OF HULL OF BARLEY IN PER CENT FROM THE FIVE VARIETIES OF BARLEY
GROWN IN THE REGIONAL SERIES IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown															Average ³
		Columbus, Ohio	East Lan- sing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmets- burg, ¹ Ia.	Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	
	1935	11.7	9.9	8.7	—	12.7	13.1	10.3	—	12.7	11.6	—	13.9	10.9	—	12.1	
	1936	—	12.2	12.5	12.0	13.2	13.2	12.9	12.5	17.8	—	12.4	8.2	9.2	7.8	11.3	
Wisconsin Barbless	1934	—	13.2	—	—	12.8	13.3	11.9	—	12.5	13.1	—	11.4	—	—	12.6	
	1935	11.7	10.5	10.8	—	9.7	10.0	11.1	11.5	15.2	12.4	12.6	8.2	8.7	8.5	10.8	
	1936	—	15.3	13.4	12.1	16.7	14.1	14.2	14.5	18.3	—	—	9.7	11.6	8.4	13.5	
Velvet	1934	—	13.4	—	—	11.4	14.8	12.5	—	11.3	11.8	—	11.8	12.9	—	12.5	
	1935	13.0	11.1	10.7	—	11.3	8.8	—	12.5	17.3	17.4	11.0	9.4	8.2	—	11.9	
	1936	—	12.6	14.0	12.2	14.2	13.6	12.8	13.2	15.2	—	—	10.8	11.6	11.1	12.8	
Manchuria	1934	—	12.9	—	—	10.3	12.9	11.2	—	11.5	11.5	—	11.1	11.3	—	11.6	
	1935	12.2	10.7	10.7	—	8.6	9.1	—	13.0	13.8	14.0	11.4	8.7	7.3	7.2	10.5	
	1936	—	12.3	10.5	10.5	12.5	12.5 ²	10.9	12.8	13.8	—	—	9.9	10.8	8.8	11.4	
Trebi	1934	—	15.7	—	—	11.4	12.9	12.0	—	16.7	13.0	—	11.4	12.1	—	13.1	
	1935	11.9	9.6	10.0	—	9.7	9.6	—	10.5	14.8	11.7	11.8	7.8	8.1	—	10.4	
	1936	—	13.5	12.7	12.6	13.9	12.5	13.5	13.5	14.8	—	—	10.9	11.8	—	13.0	

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121) in Waseca series in 1936.

³ Average for all stations; see Table XXIII for comparable averages.

TABLE IX

ENDOSPERM CHARACTER EXPRESSED AS INDEX OF MELLOWNESS IN (A) DRY BARLEY, (B) BARLEY STEEPED 24 TO 30 HOURS AT 16° C. (60.8° F.) AND DRIED, AND (C) IN THE MALT FOR THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES IN 1934, 1935, AND 1936

The index of mellowness was calculated as follows: number of steely kernels $\times 0$, number of half-steely kernels $\times \frac{1}{2}$, and number of mellow kernels $\times 1$, and sums added together

Variety	A. Dry barley B. Steeped barley C. Malt	Year	Stations where barley was grown															Average ³
			Columbus, Ohio	East Lan- sing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmettsburg, ¹ Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.		
Oder- brucker	A	1934	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	
	B	1934	—	34.5	—	—	32.5	15.5	11.5	—	29.0	11.5	—	25.5	25.5	—	23.2	
	C	1934	—	55.5	—	—	53.0	50.0	—	—	55.0	82.0	—	53.5	45.5	—	56.5	
	A	1935	74.5	72.5	85.5	—	66.5	88.5	84.0	—	84.0	85.5	—	85.0	81.5	—	85.1	
	B	1935	94.0	98.5	98.5	—	98.5	100	—	84.0	98.0	99.5	96.5	87.0	89.5	77.0	94.2	
	C	1935	96.0	98.5	96.5	—	98.0	96.0	—	92.0	87.0	89.5	98.0	94.5	97.0	97.0	95.0	
	A	1936	—	24.0	44.0	54.0	47.5	34.0	35.5	38.0	40.0	—	—	40.0	54.5	43.0	41.3	
	B	1936	—	100	99.0	100	97.5	96.0	73.0	96.0	97.5	—	—	97.0	96.0	95.5	95.2	
	C	1936	—	100	93.5	98.0	98.5	96.0	98.0	97.0	98.5	—	—	96.5	95.0	96.5	97.0	
Wis- consin Barb- less	A	1934	—	18.5	—	—	31.0	23.5	13.5	—	26.0	7.5	—	10.5	—	—	18.6	
	B	1934	—	90.0	—	—	53.5	59.5	—	—	56.0	78.0	—	48.0	—	—	64.2	
	C	1934	—	96.5	—	—	60.5	63.5	78.0	—	88.0	86.0	—	89.0	—	—	80.2	
	A	1935	69.0	53.5	78.0	—	61.5	69.0	—	70.0	57.5	63.0	47.5	30.0	44.5	43.5	57.2	
	B	1935	94.5	93.0	91.5	—	92.5	98.5	—	92.5	99.0	97.5	96.0	88.5	85.0	82.0	92.5	
	C	1935	92.5	94.5	98.5	—	94.0	98.5	—	93.0	92.0	—	96.5	93.0	87.5	97.5	94.3	
	A	1936	—	13.0	45.5	53.0	51.0	54.0	31.0	37.5	42.5	—	—	22.5	62.5	47.5	41.8	
	B	1936	—	97.0	98.5	100	97.0	98.0	92.0	95.0	100	—	—	99.0	96.0	92.5	96.8	
	C	1936	—	100	100	100	97.5	100	100	100	100	—	—	89.0	100	99.0	98.7	
Velvet	A	1934	—	34.5	—	—	26.5	17.5	13.5	—	30.5	7.0	—	12.5	20.0	—	20.2	
	B	1934	—	78.0	—	—	46.0	52.0	—	—	66.5	79.0	—	47.0	44.0	—	58.9	
	C	1934	—	95.5	—	—	63.0	75.0	81.0	—	77.5	89.5	—	94.5	80.5	—	82.1	
	A	1935	61.0	62.0	73.0	—	65.5	70.0	—	71.0	57.5	72.0	62.0	30.5	45.0	—	60.9	
	B	1935	95.5	90.0	91.5	—	96.0	98.0	—	93.0	96.5	99.0	98.0	84.5	85.5	—	93.4	
	C	1935	88.5	92.5	96.0	—	92.5	97.5	—	95.0	94.0	86.5	96.0	88.0	84.0	—	91.9	
	A	1936	—	20.0	50.5	57.0	49.0	49.0	38.5	41.5	43.0	—	—	24.0	58.5	45.5	43.3	
	B	1936	—	99.5	97.5	100	95.0	98.0	93.0	93.0	97.5	—	—	91.5	91.0	90.0	94.3	
	C	1936	—	100	100	98.0	99.5	90.0	90.0	97.0	98.0	—	—	80.5	94.5	98.0	95.0	
Man- churia	A	1934	—	35.5	—	—	47.0	18.5	25.0	—	36.0	15.5	—	8.5	26.5	—	26.4	
	B	1934	—	88.0	—	—	54.0	83.5	—	—	70.0	81.5	—	46.5	42.0	—	66.5	
	C	1934	—	88.0	—	—	81.5	83.5	88.0	—	92.5	90.5	—	87.5	85.0	—	87.1	
	A	1935	58.0	77.0	80.0	—	72.0	78.5	—	87.5	56.0	89.0	64.5	42.5	51.0	32.5	65.7	
	B	1935	94.5	96.5	90.5	—	95.5	97.0	—	97.5	99.0	99.5	98.0	97.0	96.0	67.0	94.0	
	C	1935	95.5	98.5	97.5	—	94.5	95.5	—	92.5	84.0	91.0	96.5	97.0	95.5	97.5	94.6	
	A	1936	—	23.5	49.0	53.0	48.5	29.5 ²	43.0	42.0	47.5	—	—	45.5	70.5	45.0	45.2	
	B	1936	—	96.5	97.5	99.5	99.5	78.5 ²	95.5	95.0	96.0	—	—	99.5	98.5	96.5	95.7	
	C	1936	—	96.5	99.0	100	98.5	93.5 ²	98.0	99.0	98.5	—	—	98.5	98.0	94.0	97.6	
Trebil	A	1934	—	27.5	—	—	8.5	13.5	8.0	—	21.5	5.5	—	9.0	12.5	—	13.2	
	B	1934	—	49.0	—	—	41.0	36.0	—	—	57.5	35.0	—	45.5	24.0	—	41.1	
	C	1934	—	84.0	—	—	75.8	57.5	74.0	—	82.5	78.0	—	65.0	47.0	—	70.5	
	A	1935	56.0	61.0	64.5	—	63.0	46.0	—	50.0	37.0	52.0	42.5	29.5	28.0	—	48.1	
	B	1935	73.0	88.5	97.5	—	87.0	93.0	—	87.0	86.0	88.0	88.5	73.0	65.0	—	84.2	
	C	1935	86.5	97.0	98.0	—	96.0	79.5	—	91.5	94.0	—	97.0	94.5	77.0	—	91.1	
	A	1936	—	5.0	29.0	33.0	32.0	21.0	30.5	28.5	42.0	—	—	27.5	23.0	—	27.1	
	B	1936	—	78.5	97.0	100	97.5	77.0	84.5	95.0	97.0	—	—	81.5	83.5	—	89.1	
	C	1936	—	90.0	93.0	98.0	98.5	95.0	96.5	100	95.5	—	—	74.0	76.0	—	91.6	

¹ Barleys grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121) at Waseca in 1936 series.³ Average for all stations; see Table XXIII for comparable averages.

comparable stations was slightly lower than in either of the previous years (Table XXIII). With the exception of Wisconsin Barbless, the average bushel weight based on all stations was higher in 1936 than in 1935. The detailed figures for bushel weight of barley and malt are given in Tables III and IV. Wisconsin Barbless in 1936 was lowest in bushel weight at most of the stations whereas in 1935 this variety ranked highest. The bushel weight of malts from the 1936 barleys was on the average somewhat lower than in the previous year.

TABLE X[✓]

ESTIMATED¹ TIME REQUIRED TO STEEP BARLEY TO 46 PER CENT MOISTURE CONTENT
FOR THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES
IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ⁴
		Columbus, Ohio	East Lansing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmettsburg, ² or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	Hrs. —	Hrs. 33.0	Hrs. —	Hrs. —	Hrs. 43.0	Hrs. 28.5	Hrs. 32.5	—	Hrs. 33.0	Hrs. 36.0	—	Hrs. 32.0	Hrs. 28.0	—	Hrs. 33.2
	1935	23.0	45.0	29.5	—	50.0	39.0	—	22.5	11.0	20.0	18.0	36.0	36.0	36.0	30.5
	1936	—	32.0	34.0	38.8	54.0	20.5	27.5	23.0	9.0	—	—	45.5	35.0	35.0	31.9
Wisconsin Barbless	1934	—	39.0	—	—	39.0	30.0	36.5	—	41.5	45.5	—	47.0	—	—	41.1
	1935	24.0	38.5	26.5	—	62.0	47.0	—	33.0	14.5	—	35.0	43.0	44.0	53.0	38.2
	1936	—	34.0	37.0	37.0	26.0	31.0	32.0	29.0	11.0	—	—	51.0	44.0	41.0	33.9
Velvet	1934	—	32.5	—	—	41.0	30.5	33.5	—	27.0	38.0	—	41.0	47.5	—	36.4
	1935	20.5	40.0	29.5	—	45.0	51.0	—	29.5	11.5	15.5	24.5	37.0	48.0	—	32.0
	1936	—	34.5	37.5	38.0	32.0	30.0	28.0	34.0	15.5	—	—	34.5	36.0	29.0	31.7
Manchuria	1934	—	31.0	—	—	35.0	25.5	33.0	—	39.5	28.0	—	39.0	27.0	—	32.2
	1935	18.5	24.5	23.0	—	55.0	36.0	—	22.5	11.0	13.0	22.5	41.0	38.0	39.5	28.7
	1936	—	28.0	30.5	41.5	20.0	19.0 ³	32.0	23.5	10.0	—	—	45.0	23.5	25.0	27.1
Trebil	1934	—	43.0	—	—	49.0	38.0	39.0	—	50.0	42.0	—	53.0	41.0	—	44.4
	1935	19.0	39.5	34.0	—	47.0	35.0	—	24.5	23.5	—	27.5	47.5	36.0	—	33.3
	1936	—	56.0	55.0	52.0	36.5	27.5	48.5	33.0	19.0	—	—	37.0	35.0	—	39.0

¹ Hours were estimated by using the logarithmic graph for water absorption.

² Barleys grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

³ Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121) in the Waseca series in 1936.

⁴ Average for all stations; see Table XXIII for comparable averages.

The kernel weight of barleys and malts in 1936 was somewhat low but for all stations not appreciably lower than in 1935. (See Table XXIII.) However, the kernel weight of both barley and malt was reduced considerably at some stations, including Urbana, Illinois, Madison, Wisconsin, Waseca, Minnesota, and Kanawha, Iowa. There was also a slight reduction in kernel weight in some of the barleys and malts from Bozeman, Montana, Fort Collins, Colorado, and Davis, California. The data on kernel weight of barleys and malts are given in Tables V and VI. The five varieties have maintained the same relative order for kernel weight in the three years.

The data on skinned and broken kernels show relatively little varietal difference in 1936 or the two previous years. The general conclusion that the smooth-awned barley varieties have been more severely damaged by skinning has not been clearly substantiated by the data from the barleys grown at the stations cooperating in the

TABLE XI

TIME STEEPED IN WATER HELD AT 16° C. (60.8° F.) AND MOISTURE CONTENT OF BARLEY, AFTER STEEPING FOR THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES IN 1934, 1935, AND 1936. STEEP WATER CHANGED EVERY EIGHT HOURS

Variety	Year	A. Time steeped, hrs.	B. Moisture content, %	Location where barley was grown														Average ³
				Columbus, Ohio	East Lansing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmetsburg, ¹ or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	A hrs.	%	—	34	—	—	36	34	41	—	32	35	—	35	35	—	34
	1934	B %	%	—	46.2	—	—	44.9	46.9	47.5	—	45.8	45.9	—	46.7	47.5	—	46.4
	1935	A hrs.	%	43	37	40	—	40	35	—	30	18	18	30	40	42	42	35
	1935	B %	%	49.7	44.9	47.9	—	44.6	45.4	—	47.8	51.9	45.7	49.2	46.7	40.8	47.0	47.3
	1936	A hrs.	%	—	42	47	45	41	32	36	32	15	—	—	41	34	41	37
	1936	B %	%	—	47.6	47.9	46.9	44.2	48.4	47.5	47.9	48.8	—	—	46.4	45.8	47.1	47.1
Wisconsin Barbless	1934	A hrs.	%	—	40	—	—	36	38	44	—	40	42	—	47	—	—	41
	1934	B %	%	—	46.2	—	—	45.5	45.9	47.0	—	45.8	45.6	—	46.0	—	—	46.0
	1935	A hrs.	%	47	46	48	—	48	48	—	46	24	24	42	47	45	47	44
	1935	B %	%	49.5	47.2	48.7	—	44.3	46.1	—	47.9	48.3	—	47.1	46.4	46.2	45.2	47.0
	1936	A hrs.	%	—	44	45	44	35	47	53	38	15	—	—	60	45	41	43
	1936	B %	%	—	47.5	47.1	47.3	47.7	48.4	49.0	47.5	47.9	—	—	47.3	46.2	46.0	47.5
Velvet	1934	A hrs.	%	—	38	—	—	32	34	41	—	32	38	—	40	41	—	37
	1934	B %	%	—	46.9	—	—	44.9	46.7	47.3	—	46.9	46.0	—	45.9	44.9	—	46.2
	1935	A hrs.	%	40	37	40	—	42	43	—	37	20	24	33	44	40	—	35
	1935	B %	%	49.9	45.6	48.0	—	45.6	45.1	—	47.2	49.6	48.6	47.8	47.2	43.0	—	47.0
	1936	A hrs.	%	—	43	47	47	35	35	48	38	19	—	—	48	35	32	39
	1936	B %	%	—	47.2	47.5	47.5	46.6	46.9	49.4	46.8	48.1	—	—	46.4	45.9	46.6	47.2
Manchuria	1934	A hrs.	%	—	32	—	—	28	30	40	—	38	34	—	37	38	—	35
	1934	B %	%	—	46.2	—	—	45.0	46.9	47.3	—	45.8	46.9	—	45.6	47.9	—	46.4
	1935	A hrs.	%	32	30	40	—	38	30	—	30	19	22	21	37	29	42	31
	1935	B %	%	49.5	46.9	49.1	—	44.1	45.1	—	47.6	49.5	49.4	45.6	45.3	44.3	46.4	46.9
	1936	A hrs.	%	—	38	42	44	33	32	42	32	19	—	—	41	32	32	35
	1936	B %	%	—	48.0	47.7	46.4	48.2	49.3 ²	48.3	47.7	50.5	—	—	47.3	48.0	47.5	48.1
Treibi	1934	A hrs.	%	—	48	—	—	36	38	44	—	60	50	—	50	58	—	47
	1934	B %	%	—	46.7	—	—	44.7	46.0	46.7	—	47.4	47.0	—	45.6	47.9	—	46.5
	1935	A hrs.	%	42	46	48	—	48	48	—	37	30	—	37	46	47	—	43
	1935	B %	%	51.1	46.9	47.8	—	46.2	47.9	—	48.5	47.3	—	48.0	45.7	47.9	—	47.7
	1936	A hrs.	%	—	41	62	66	44	35	69	43	29	—	—	47	47	—	48
	1936	B %	%	—	44.3	46.9	47.7	47.3	47.5	49.3	47.9	49.6	—	—	46.6	47.1	—	47.4

¹ Barleys grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121) in Waseca series in 1936.

³ Average for all stations; see Table XXIII for comparable averages.

regional barley studies. These results would indicate that with careful threshing varieties differ only slightly in damage from skinning and breaking, although under farm and industrial handling the general survey data obtained in another study show that more skinning occurs in the smooth-awned barleys. In 1936, however, records obtained from over 800 farm samples of Wisconsin Barbless and Oderbrucker

TABLE XII¹
 MOISTURE CONTENT OF GREEN MALT AT THE END OF THE GERMINATION PERIOD
 FOR THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL
 SERIES IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ²
		Columbus, Ohio	East Lams- sing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmets- burg ¹ or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
	1935	46.3	52.4	—	—	53.2	51.2	48.8	—	52.0	50.4	—	49.5	50.8	—	51.0
	1936	—	46.1	45.7	—	45.2	46.2	—	49.1	51.1	44.9	44.0	44.3	44.2	48.5	46.3
Wisconsin Barbless	1934	—	49.9	—	—	52.9	50.3	48.6	—	51.4	50.9	—	51.0	—	—	50.7
	1935	44.6	46.1	46.2	45.9	46.7	45.8	—	46.8	44.0	—	43.3	43.3	43.0	44.2	44.8
	1936	—	46.4	44.5	47.4	45.4	44.1	47.1	46.6	46.5	—	—	45.1	46.0	45.7	45.9
Velvet	1934	—	50.7	—	—	52.0	50.5	48.9	—	51.3	50.7	—	49.9	51.3	—	50.7
	1935	47.4	45.8	46.4	—	47.7	46.3	—	47.3	47.1	45.8	43.9	45.4	44.5	—	46.1
	1936	—	45.4	46.1	44.6	46.2	45.3	46.4	47.6	45.6	—	—	44.7	46.8	44.1	45.7
Manchuria	1934	—	52.1	—	—	54.0	52.7	49.2	—	51.1	52.5	—	50.9	50.7	—	51.6
	1935	47.5	46.0	49.1	—	45.8	46.7	—	48.2	48.0	46.5	44.6	45.0	44.2	43.5	46.3
	1936	—	45.6	46.0	46.0	46.1 ²	48.2	46.7	46.9	47.3	—	—	48.4	45.2	51.3	47.1
Trebti	1934	—	50.1	—	—	52.4	51.2	48.1	—	49.9	49.4	—	49.4	49.1	—	49.9
	1935	46.9	45.9	49.0	—	47.9	44.5	—	48.1	45.6	—	45.9	45.0	43.4	—	46.2
	1936	—	48.7	44.9	44.8	44.7	44.7	45.4	44.7	49.0	—	—	45.1	45.0	—	45.8

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121) in the Waseca series in 1936.

³ Average for all stations; see Table XXIII for comparable averages.

TABLE XIII¹
 RECOVERY OF MALT FROM BARLEY ON THE DRY BASIS FOR THE FIVE VARIETIES
 OF BARLEY GROWN IN THE REGIONAL SERIES IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ²
		Columbus, Ohio	East Lams- sing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmets- burg ¹ or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
	1935	88.5	85.9	83.2	—	83.7	83.3	90.4	—	86.5	88.8	—	90.1	88.4	—	87.8
	1936	—	90.2	87.6	90.4	85.7	87.7	89.4	88.9	86.6	—	—	85.9	87.9	84.6	87.7
Wisconsin Barbless	1934	—	89.5	—	—	84.1	89.3	90.4	—	87.9	88.2	—	89.1	—	—	88.4
	1935	85.4	89.8	86.5	—	88.3	90.2	—	85.4	87.8	—	91.7	88.4	86.5	86.6	87.9
	1936	—	92.6	90.8	91.9	90.5	90.5	87.7	89.5	88.5	—	—	89.8	89.7	88.4	90.0
Velvet	1934	—	88.9	—	—	84.7	88.4	90.0	—	87.5	88.0	—	90.0	87.4	—	88.1
	1935	85.1	85.2	87.6	—	89.0	89.7	—	81.9	83.9	86.6	89.4	91.0	84.6	—	86.4
	1936	—	89.4	89.7	91.0	89.5	89.2	86.8	87.9	90.9	—	—	91.3	87.3	90.1	89.4
Manchuria	1934	—	86.0	—	—	82.3	81.3	88.8	—	86.5	84.9	—	88.8	88.6	—	85.9
	1935	84.1	86.5	83.7	—	87.0	85.2	—	79.5	80.0	89.2	90.3	88.9	86.7	88.0	85.5
	1936	—	89.3	89.6	89.6	90.9	87.3 ²	88.7	88.8	89.1	—	—	86.3	88.3	86.1	88.6
Trebti	1934	—	90.1	—	—	84.1	89.5	91.6	—	90.3	90.4	—	91.3	91.4	—	89.8
	1935	90.0	89.7	86.2	—	85.4	94.3	—	83.9	85.0	—	86.8	89.2	88.2	—	87.9
	1936	—	88.6	91.0	90.9	92.2	90.2	90.8	90.7	88.5	—	—	92.5	89.1	—	90.4

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

² Manchuria (Minn. 184) was substituted for Manchuria (N. Dak. 2121) in the Waseca series in 1936.

³ Average for all stations; see Table XXIII for comparable averages.

TABLE XIV
ASH CONTENT OF FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES
IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ³
		Columbus, Ohio	East Lansing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmets- burg, ¹ or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
	1935	4.34	2.97	3.15	—	2.60	2.65	2.62	—	2.77	2.49	—	2.41	2.32	—	2.49
	1936	—	2.70	2.88	3.14	2.82	2.76	2.95	3.40 ¹	2.96	3.20	3.31	2.80	2.57	2.73	3.21
Wisconsin Barbless	1934	—	1.73	—	—	2.74	2.59	2.55	—	2.54	2.35	—	2.30	—	—	2.40
	1935	3.12	2.79	2.97	—	3.10	3.01	—	3.10	3.19	3.17	3.07	2.63	2.57	—	2.80
	1936	—	2.43	2.86	2.94	3.07	2.69	2.94	3.00	3.01	—	—	2.85	2.70	2.81	2.85
Velvet	1934	—	1.86	—	—	2.40	2.62	2.56	—	2.54	2.34	—	2.37	—	—	2.36
	1935	3.43	2.95	3.22	—	3.43	3.25	—	3.65	3.61	3.53	3.34	2.77	2.42	—	3.22
	1936	—	2.99	2.87	3.07	2.99	2.62	2.83	3.00	2.74	—	—	2.85	2.61	2.95	2.91
Manchuria	1934	—	1.72	—	—	2.31	2.47	2.44	—	2.55	2.41	—	2.40	2.27	—	2.32
	1935	3.15	3.11	3.32	—	3.36	3.37	—	3.40	3.66	3.59	3.41	2.83	2.58	2.89	3.22
	1936	—	2.78	2.81	2.90	2.91	2.56 ²	2.84	3.10	2.78	—	—	2.98	2.74	2.91	2.85
Trebti	1934	—	1.86	—	—	2.12	2.37	2.63	—	1.94	2.10	—	2.07	2.07	—	2.14
	1935	3.25	2.92	2.92	—	3.08	2.91	—	3.15	3.36	3.15	3.14	2.59	2.57	—	3.00
	1936	—	2.46	2.74	2.74	2.81	2.38	2.85	2.80	2.58	—	—	2.93	2.68	—	2.70

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.² Manchuria (Minn. 184) was substituted for Manchuria (N. Dak. 2121) in Waseca series in 1936.³ Average for all stations; see Table XXIII for comparable averages.

TABLE XV
YIELD OF EXTRACT ON THE DRY BASIS OF FINE GRIND MALT. FIVE VARIETIES
OF BARLEY GROWN IN THE REGIONAL SERIES FOR 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ³
		Columbus, Ohio	East Lansing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmets- burg, ¹ or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
	1935	73.2	74.6	75.6	—	71.0	71.4	72.6	—	72.0	70.0	—	72.8	70.9	—	71.5
	1936	—	74.0	73.0	75.7	72.1	71.7	70.7	71.0 ¹	65.7	67.4	72.6	73.6	74.7	77.2	73.0
Wisconsin Barbless	1934	—	69.9	—	—	67.9	68.5	70.9	—	71.2	67.8	—	71.4	—	—	69.7
	1935	71.3	72.4	74.2	—	74.2	72.4	—	71.0	63.8	—	71.8	72.3	75.0	76.6	72.3
	1936	—	69.1	70.4	73.4	68.4	70.3	68.8	68.7	62.6	—	—	72.9	72.9	74.8	70.2
Velvet	1934	—	70.7	—	—	69.5	69.2	71.4	—	72.4	70.5	—	74.0	69.6	—	70.9
	1935	71.9	78.8	76.0	—	76.1	75.2	—	72.6	63.4	68.0	73.4	72.3	73.6	76.0	72.4
	1936	—	73.3	72.6	75.8	72.0	70.7	70.9	71.6	66.8	—	—	71.6	75.8	75.3	72.4
Manchuria	1934	—	70.9	—	—	73.1	72.4	72.0	—	74.3	71.7	—	73.8	70.3	—	72.3
	1935	72.6	73.4	75.9	—	77.0	76.0	—	72.7	66.3	69.7	72.4	74.2	77.2	77.5	73.7
	1936	—	73.0	72.6	76.4	72.2	72.3 ²	73.1	71.5	67.0	—	—	74.9	75.4	75.6	73.1
Trebti	1934	—	73.6	—	—	69.3	69.3	73.7	—	73.9	72.5	—	74.7	70.8	—	72.2
	1935	71.9	75.0	77.1	—	77.2	74.4	—	70.9	66.5	—	72.4	74.9	75.5	—	73.6
	1936	—	69.9	74.9	76.9	73.1	72.8	71.9	71.9	66.0	—	—	72.6	73.7	—	72.4

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121) as latter not grown at Waseca with series in 1936.³ Average for all stations; see Table XXIII for comparable averages.

show less than 0.3% difference between the two varieties in skinning and breaking. The data on skinned and broken kernels are given in Table VII.

The percentage of hull in the barleys grown in 1936 was in general like that of 1934, though higher than in 1935. The differences in hull percentage for the three years were principally in the barleys grown at the stations in the upper Mississippi Valley area where the seasonal contrast of the three years was much greater than at the irrigated stations. The three varieties, Wisconsin Barbless, Velvet, and Trebi, have averaged slightly more than Oderbrucker and Manchuria in

TABLE XVI

TOTAL PROTEIN CONTENT OF THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES FOR 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ³
		Columbus, Ohio	East Lan- sing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmets- burg ¹ or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oder- brucker	1934	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
	1935	14.49	14.05	14.18	—	20.08	15.53	14.36	—	17.15	17.97	—	16.61	—	—	17.95
	1936	—	11.80	13.03	10.98	13.10	16.00	15.47	16.16 ¹	16.75	15.18	14.00	15.80	15.53	13.16	14.93
Wis- consin Barbless	1934	—	—	—	—	19.68	—	15.84	—	17.65	—	—	15.56	—	—	17.18
	1935	14.08	13.88	13.03	—	12.64	14.27	—	15.41	16.00	14.64	13.34	14.80	14.23	12.70	14.08
	1936	—	13.56	12.51	10.57	12.89	12.66	13.74	13.44	15.74	—	—	14.43	13.73	14.37	13.42
Velvet	1934	—	—	—	—	20.02	—	16.84	—	17.15	—	—	15.03	—	—	17.26
	1935	14.70	15.59	13.47	—	13.31	13.53	—	15.55	16.49	14.70	14.02	14.55	14.93	—	14.62
	1936	—	11.90	13.00	11.58	13.59	16.56	15.52	14.43	16.34	—	—	14.61	14.18	15.12	14.20
Man- churia	1934	—	—	—	—	16.72	—	15.90	—	16.33	—	—	15.24	—	—	16.05
	1935	15.80	15.12	14.04	—	13.65	15.34	—	16.24	17.06	15.49	15.74	14.90	14.93	12.13	15.04
	1936	—	12.11	13.25	11.17	13.62	17.18 ²	14.43	14.49	15.10	—	—	14.52	13.68	14.18	13.97
Trebi	1934	—	—	—	—	17.34	—	14.12	—	14.33	—	—	13.34	—	—	14.78
	1935	15.09	12.16	11.39	—	11.26	13.07	—	16.00	15.74	15.96	13.80	13.59	14.42	—	13.86
	1936	—	14.67	11.42	9.70	11.45	15.25	13.47	12.98	15.07	—	—	14.46	12.98	—	13.14

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121), as latter not grown at Waseca with series in 1936.

³ Average for all stations; see Table XXIII for comparable averages.

hull content in the comparative three-year average (Tables VIII and XXIII).

The endosperm texture of the barleys grown at the various stations in 1936 was decidedly hard although the starch mass became mellow after steeping and drying. The starch mass of the dry kernels was nearly as hard as in the barleys grown in 1934. Steeping the barley for 24 to 30 hours at 16° C. (60.8° F.) followed by drying resulted in an average mellowness slightly better than that obtained with the relatively mellow barley of 1935 and much better than in 1934 (Tables IX and XXIII). The Trebi variety was lowest in mellow kernels

TABLE XVII

TOTAL PROTEIN CONTENT OF THE MALTS FROM THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ¹			
		Columbus, Ohio	East Lansing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmettsburg ¹ or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.				
Oderbrucker	1934	%	15.53	%	—	%	20.73 ³	%	19.03	%	16.09 ³	%	17.72 ³	%	16.40 ³	%	16.94	%	17.45 ³
	1935	14.18	13.67	14.31	—	13.22	14.09	—	17.12	17.24	14.36	13.68	15.61	14.84	12.89	14.60	—	14.60	
	1936	—	11.55	12.43	10.52	12.98	15.53	15.38	14.49	17.93	—	—	15.28	14.61	13.72	14.04	—	14.04	
Wisconsin Barbless	1934	—	14.44	—	—	19.84	18.13	15.40	—	15.87	16.34	—	15.55	—	—	—	—	16.51	
	1935	13.62	13.35	12.57	—	12.24	13.62	—	14.24	15.44	—	12.79	14.43	13.59	12.16	13.46	—	13.46	
	1936	—	13.29	11.70	9.64	12.35	14.70	13.61	13.03	15.13	—	—	14.34	13.38	14.12	13.21	—	13.21	
Velvet	1934	—	15.54	—	—	20.27	18.96	16.75	—	17.54	16.18	—	14.00	16.90	—	—	—	17.02	
	1935	14.15	15.03	13.04	—	12.64	13.06	—	15.47	16.39	14.22	13.90	14.08	14.67	—	14.24	—	14.24	
	1936	—	11.35	12.70	11.01	12.63	15.96	15.13	13.99	16.09	—	—	14.37	13.83	15.16	13.84	—	13.84	
Manchuria	1934	—	17.43	—	—	17.80	17.22	15.87	—	15.68	15.71	—	15.12	16.33	—	—	—	16.39	
	1935	15.53	14.71	13.80	—	13.56	14.84	—	15.28	16.82	15.81	15.87	14.12	14.67	11.46	14.70	—	14.70	
	1936	—	12.11	13.32	10.83	13.13	16.00 ²	14.12	14.31	15.71	—	—	14.02	15.28	14.02	13.90	—	13.90	
Trebis	1934	—	12.91	—	—	18.09	16.43	14.30	—	13.56	13.22	—	13.22	15.81	—	—	—	14.69	
	1935	14.64	11.58	11.08	—	10.88	13.16	—	15.47	15.75	—	13.64	12.60	14.58	—	13.34	—	13.34	
	1936	—	14.37	11.29	9.17	10.83	15.44	13.64	12.63	16.00	—	—	14.84	12.56	—	13.08	—	13.08	

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.² Manchuria (Minn. 184) substituted for Manchuria (N. Dak. 2121) as latter not grown at Waseca with series in 1936.³ Protein content of the four malts from barleys in which protein was determined in 1934 is as follows: Oderbrucker, 17.73, Wisconsin Barbless, 16.86, Velvet, 17.14, Manchuria, 16.11, and Trebis, 14.79%.⁴ Average for all stations; see Table XXIII for comparable averages.

both before and after steeping. The seasonal effect on mellowness was seemingly greater than either station or variety although the influence of the latter was very significant. Modification of the endosperm mass in malting was very good in all varieties except Trebi, and Wisconsin Barbless in 1934.

The average rate of water absorption in the 1936 crop was somewhat slower for Oderbrucker and Trebi and slightly faster for Velvet, Manchuria, and Wisconsin Barbless than in the 1935 crop. Over the

TABLE XVIII[✓]

SOLUBLE NITROGEN IN THE WORT EXPRESSED AS PROTEIN ($N \times 6.25$) FOR THE MALTS FROM THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ³
		Columbus, Ohio	East Lansing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmettsburg, ¹ or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934	—	5.06	—	—	7.28	6.31	5.62	—	5.84	5.31	—	4.68	5.12	—	5.65
	1935	5.06	4.82	5.12	—	4.75	5.12	—	7.12	7.25	4.50	5.97	4.28	4.69	4.62	5.27
	1936	—	3.79	4.44	3.94	4.84	5.58	5.35	5.21	5.55	—	—	5.04	5.23	5.25	4.93
Wisconsin Barbless	1934	—	3.47	—	—	5.37	4.65	4.03	—	4.25	3.93	—	3.47	—	—	4.17
	1935	4.12	4.03	4.00	—	3.75	3.87	—	4.65	4.62	—	4.03	3.06	3.53	3.18	3.89
	1936	—	3.57	3.37	3.19	3.71	3.83	3.90	3.55	4.23	—	—	3.25	3.91	3.85	3.67
Velvet	1934	—	4.71	—	—	6.25	6.03	5.19	—	5.53	4.78	—	4.25	4.78	—	5.19
	1935	5.97	5.00	4.97	—	4.97	4.92	—	6.19	6.44	6.16	5.44	3.31	4.88	—	5.20
	1936	—	4.05	4.60	4.23	4.55	5.31	5.24	5.02	5.39	—	—	3.49	5.01	4.94	4.71
Manchuria	1934	—	5.09	—	—	6.22	5.81	5.47	—	5.28	4.59	—	4.24	4.72	—	5.18
	1935	6.78	5.53	4.81	—	5.62	5.97	—	7.57	7.12	7.25	6.90	3.93	5.25	3.93	5.89
	1936	—	4.18	4.60	3.93	4.68	5.63 ²	5.13	4.90	7.35	—	—	5.03	4.43	5.47	5.04
Trebi	1934	—	3.62	—	—	4.81	4.47	3.93	—	3.78	3.34	—	3.18	3.53	—	3.83
	1935	5.53	3.90	4.18	—	3.78	2.94	—	5.47	4.85	—	4.65	4.00	4.15	—	4.34
	1936	—	3.99	—	3.27	3.02	3.35	4.74	3.83	4.90	—	—	3.49	3.54	—	3.78

¹ Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

² Manchuria (Minn. 184) was substituted for Manchuria (N. Dak. 2121), in the Waseca series in 1936 as the latter was not grown.

³ Average for all stations; see Table XXIII for comparable averages.

three-year period at six comparable stations Trebi required the longest time for absorption, with Wisconsin Barbless next, and Velvet, Oderbrucker and Manchuria following in the order given. The Wisconsin Barbless samples grown at the stations in the upper Mississippi Valley area in 1936 reached the same average moisture content as in 1935 with an average of six hours less time in the steep than in 1935. The immaturity of the grain and the lower kernel weight were largely responsible for this. The other varieties showed this seasonal influence on water absorption at only part of the stations (Table X).

The barleys were steeped and malted at approximately the same

moisture content as in 1935. The average moisture content out of the steep for 1934 was 46.5%, for 1935, 47.2%, and for 1936, 47.5%. The average moisture content at the end of the germination period for 1935 was 45.9%, and for 1936, 46.3% (Tables XI and XII). The 1934 barleys were grown at a higher moisture content especially toward the

TABLE XIX ✓

THE COMPARISON OF AVERAGE NITROGEN MODIFICATION IN THE WORTS, AVERAGE KERNEL WEIGHT OF MALT, AVERAGE RECOVERY OF MALT FROM BARLEY AND AVERAGE DIASTATIC POWER OF MALTS FOR THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES IN 1934, 1935, AND 1936

Variety of barley	Year grown	Total samples averaged, ¹ number	Kernel weight of malt, dry basis, in mg.	Malt nitrogen, %	Soluble nitrogen in wort, %	Soluble nitrogen as % of malt nitrogen, %	Recovery of malt from barley, dry basis, %	Dia-static power of malt, °L.
Oderbrucker	1934	8	25.6	2.79	.904	32.40	87.8	153.1
	1935	12	22.6	2.34	.843	36.03	85.2	182.1
	1936	11	23.0	2.25	.789	35.07	87.7	191.9
3 year average		31	23.7	2.46	.845	34.51	86.9	175.7
Wisconsin Barbless	1934	7	26.0	2.64	.667	25.27	88.4	77.6
	1935	11	25.0	2.15	.622	28.93	87.9	133.8
	1936	11	24.2	2.11	.588	28.01	90.0	117.6
3 year average		29	25.1	2.30	.626	27.40	88.8	109.7
Velvet	1934	8	24.6	2.72	.830	30.51	88.1	105.3
	1935	11	24.6	2.28	.846	37.11	86.4	165.4
	1936	11	23.1	2.22	.755	34.26	89.4	152.7
3 year average		30	24.1	2.40	.810	33.96	88.0	141.1
Manchuria	1934	8	22.9	2.62	.829	36.65	85.9	124.7
	1935	12	21.2	2.35	.942	40.09	85.5	190.8
	1936	11	21.6	2.32	.778	35.04	88.6	194.5
3 year average		31	21.9	2.40	.850	37.26	86.7	170.0
Trebí	1934	8	32.6	2.35	.613	26.08	89.8	75.9
	1935	10	28.7	2.13	.694	32.58	87.9	143.5
	1936	10	29.6	2.09	.605	29.06	90.4	158.3
3 year average		28	30.3	2.19	.637	29.24	89.4	125.9

¹ Average of all malts for each year, therefore not comparable averages between years.

end of the germination period. The writers believe the moistures used have been high enough to give satisfactory results with all five varieties and thus have avoided the use of different moistures for each variety in a comparative study of this type. The physiological studies on moisture in relation to malting indicate that Wisconsin Barbless is more tolerant of higher moisture content in malting than Oderbrucker.

The average recovery of malt from barley was slightly higher in 1936 than in 1935, and considerably more than in 1934. The recovery from the Oderbrucker variety from three stations was lower than in 1935. The data on malt recovery are given in Tables XIII and XXIII. Years, varieties and stations affect malt recovery in descending order.

The percentage of ash in the 1936 barleys from all stations except the three western locations was lower than in 1935. The influence of the season on ash content was greater than was the location where grown or the variety. The data on ash content for the three years are given in Tables XIV and XXIII.

TABLE XX

THE COMPARISON OF AVERAGE NITROGEN MODIFICATION IN THE WORTS, AND CERTAIN PHYSICAL AND CHEMICAL FACTORS OF BARLEY AND MALT FOR THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES IN 1936

	Barley variety				
	Oder-brucker	Wisconsin Barbless	Velvet	Man-churia	Trebi
Total number of malts analyzed	11	11	11	11	10
Kernel weight of barley, dry basis, in mg.	26.0	26.7	25.8	24.7	33.3
Nitrogen in barley, %	2.27	2.09	2.28	2.19	2.10
Index of mellowness of dry barley, %	41.3	41.8	43.3	45.2	27.1
Index of mellowness of barley steeped and dried, %	95.2	96.8	94.3	95.7	89.1
Kernel weight of malt, dry basis, in mg.	23.0	24.2	23.2	21.6	29.6
Nitrogen in malt, %	2.25	2.11	2.22	2.32	2.09
Soluble nitrogen in wort, %	.789	.588	.755	.778	.605
Soluble nitrogen as % of malt N., %	32.25	28.01	34.26	35.04	29.06
Permanently soluble nitrogen in wort, %	.748	.564	.724	.748	.574
Permanently soluble nitrogen as % of malt N., %	33.27	26.73	32.61	33.99	27.59
Permanently soluble nitrogen as % of wort N., %	94.8	95.9	95.9	95.4	94.9
Formol nitrogen in wort, %	.151	.112	.150	.158	.104
Formol nitrogen as % of malt N., %	6.73	5.31	6.76	6.81	4.98
Formol nitrogen as % of wort N., %	19.11	17.36	19.79	20.32	17.17
Formol nitrogen as % of permanently soluble N., %	20.24	19.86	20.72	20.70	18.09
Recovery of malt from barley, dry basis, %	87.7	90.0	89.4	88.6	90.4
Index of mellowness of malt, %	97.0	98.7	95.0	97.6	91.6
Diastatic power of malt, °L.	192	118	153	194	158

The extract yield of the malts was low especially in barleys grown at most of the stations in the upper Mississippi Valley. The average extract yield for all stations was significantly lower than in 1935 for the Wisconsin Barbless and Trebi varieties and about the same as in 1935 for Oderbrucker, Velvet, and Manchuria. The average extract yield for the malts from the six comparable stations was lower than the malts from either of the previous seasons (Table XXIII). The barleys grown at one station in the north-central area, namely DeKalb, Illinois, produced malts very high in extract content. In general the malts from Wisconsin Barbless barleys grown at the stations in the upper Mississippi Valley were low in extract. The relative position of the varieties on the basis of average extract yield from the malts shifted somewhat from the 1934 and 1935 rankings in that Oderbrucker

TABLE XXI

COMPARISON OF NITROGEN MODIFICATION IN THE LABORATORY WORTS AND KERNEL WEIGHT OF MALT, RECOVERY OF MALT FROM BARLEY, DIASTATIC POWER OF THE MALTS AND ENDSPERM CHARACTER OF THE MALTS FROM THE BARLEY VARIETIES GROWN IN THE REGIONAL SERIES IN 1936

Variety and location where barley was grown	Moisture content of malt	Kernel weight of malt, bush	Nitrogen in malt	Soluble nitrogen in wort	Soluble nitrogen as % of malt N	Permanently soluble nitrogen in wort	Permanently soluble nitrogen as % of malt N	Permanently soluble nitrogen in wort N	Formol nitrogen in wort	Formol N as % of malt N	Formol N as % of permanent soluble N	Recovery of malt from barley, dry basis	Dia- static power of malt	Endsperm character of malt, f mellowness
	%	Mg.	%	%	%	%	%	%	%	%	%	%	%	%
Oderbrucker	5.2	26.4	1.85	.606	32.76	.579	31.29	95.5	.113	6.11	18.64	19.52	149	100
East Lansing, Mich.	4.1	24.7	1.99	.712	35.78	.679	34.12	95.4	.135	6.79	18.96	19.88	168	93.5
Urbana, Ill.	4.0	25.7	1.68	.631	37.56	.603	35.89	95.6	.111	6.61	17.59	18.41	113	98.0
DeKalb, Ill.	5.6	20.7	2.08	.775	37.26	.743	35.72	95.9	.140	6.73	18.06	18.84	182	98.5
Madison, Wis.	4.5	20.8	2.49	.893	35.86	.845	33.94	94.6	.178	7.15	19.93	21.06	212	96.0
Waseca, Minn.	4.2	20.7	2.46	.856	34.80	.814	33.09	95.1	.154	6.26	17.99	18.92	207	98.0
Kanawha, Ia.	4.2	20.4	2.32	.834	35.95	.797	34.35	95.6	.158	6.81	18.94	18.80	197	97.0
Ames, Ia.	3.3	16.1	2.87	.888	30.94	.839	29.23	94.5	.188	6.55	21.17	22.40	182	98.5
Brookings, S. D.	5.6	26.7	2.45	.807	35.73	.751	30.65	93.1	.150	6.12	18.59	19.97	272	96.5
Bozeman, Mont.	4.8	25.0	2.34	.836	35.73	.789	33.72	94.4	.170	7.26	20.33	21.55	215	95.0
Fort Collins, Col.	6.4	26.4	2.20	.839	38.14	.789	35.86	94.0	.168	7.64	20.02	21.29	214	96.5
Davis, Cal.														
Oderbrucker, average	4.7	23.0	2.25	.789	35.25	.748	33.27	94.8	.151	6.73	19.11	20.24	192	97.0
Wisconsin Barbless														
East Lansing, Mich.	4.5	32.4	2.13	.571	26.81	.550	25.82	96.3	.094	4.41	16.46	17.09	119	100
Urbana, Ill.	4.1	23.4	1.87	.540	28.88	.515	27.54	95.4	.088	4.71	16.30	17.09	116	100
DeKalb, Ill.	4.4	22.9	1.54	.512	33.25	.491	31.88	95.9	.089	5.78	17.38	18.13	74	100
Madison, Wis.	4.4	21.2	1.98	.594	30.00	.575	29.04	96.8	.117	5.91	19.70	20.35	89	97.5
Waseca, Minn.	4.0	20.5	2.36	.613	25.97	.587	24.87	95.8	.098	4.15	15.99	16.69	110	100
Kanawha, Ia.	4.1	22.3	2.18	.626	28.72	.603	27.66	96.3	.106	4.86	16.93	17.58	121	100
Ames, Ia.	5.0	21.6	2.09	.568	27.18	.542	25.93	95.4	.099	4.74	17.43	18.27	124	100
Brookings, S. D.	3.5	16.3	2.42	.679	28.06	.648	26.78	95.4	.139	5.74	20.47	21.45	104	100
Bozeman, Mont.	5.2	31.9	2.30	.521	22.65	.497	21.61	95.4	.081	3.52	15.55	16.30	155	89.0
Fort Collins, Col.	5.3	24.5	2.14	.627	29.30	.603	28.18	96.2	.108	5.05	17.22	17.91	129	100
Davis, Cal.	5.7	29.5	2.26	.617	27.30	.594	26.28	96.3	.108	4.78	17.50	18.18	153	99.0
Wisconsin Barbless, average	4.6	24.2	2.11	.588	28.01	.564	26.73	95.9	.112	5.31	17.36	19.86	118	98.7

TABLE XXI—Continued

Variety and location where barley was grown	Moisture content of malt	Kernel weight of malt, dry basis	Nitrogen in malt	Soluble nitrogen in wort	Soluble nitrogen as % of malt N	Permanently soluble nitrogen in wort	Permanently soluble nitrogen as % of malt N	Permanently soluble nitrogen as % of wort N	Formol nitrogen in wort	Formol N as % of malt N	Formol N as % of wort N	Formol N as % of permanent soluble N	Recovery of malt from barley, dry basis	Diastatic power of malt	Endosperm character of malt. Index of mellowness
	%	Mp.	%	%	%	%	%	%	%	%	%	%	%	%L.	%
Velvet	5.1	24.5	1.82	.649	35.66	.626	34.40	96.5	.123	6.76	18.95	19.65	89.4	118	100
East Lansing, Mich.	4.3	23.5	2.03	.737	36.31	.702	34.58	95.2	.140	6.90	19.00	19.94	89.7	150	100
Urbana, Ill.	4.4	25.0	1.76	.679	38.58	.655	37.22	96.5	.135	7.67	19.88	20.61	91.0	95	98.0
DeKalb, Ill.	4.6	20.4	2.02	.728	36.04	.703	34.80	96.6	.154	7.62	21.15	21.91	89.5	123	99.5
Madison, Wis.	4.4	22.1	2.56	.851	33.24	.818	31.95	96.1	.175	6.84	20.56	21.39	89.2	171	90.0
Waseca, Minn.	4.2	20.7	2.42	.839	34.67	.808	33.39	96.3	.163	6.73	19.42	20.17	86.8	165	90.0
Kanawha, Ia.	5.2	22.1	2.24	.804	35.89	.769	34.34	95.6	.155	6.92	19.28	20.16	87.9	170	97.0
Ames, Ia.	4.0	17.4	2.58	.863	33.45	.832	32.25	96.4	.183	7.09	21.21	22.00	90.9	160	98.0
Brookings, S. D.	5.0	29.0	2.30	.859	24.30	.830	23.04	94.8	.089	3.87	15.92	16.79	91.3	162	80.5
Bozeman, Mont.	5.0	24.3	2.22	.802	36.13	.770	34.68	96.0	.176	7.93	21.95	22.86	87.3	175	94.5
Fort Collins, Col.	5.3	25.8	2.43	.791	32.55	.749	30.82	94.7	.161	6.63	20.35	21.50	90.1	191	98.0
Davis, Cal.															
Velvet, average	4.7	23.3	2.22	.755	34.26	.724	32.61	95.9	.150	6.76	19.79	20.72	89.4	153	95.0
Manchuria															
East Lansing, Mich.	5.2	23.3	1.94	.670	34.54	.642	33.09	95.8	.118	6.08	17.61	18.38	89.8	158	96.5
Urbana, Ill.	4.9	22.3	2.13	.733	34.41	.702	32.96	95.8	.136	6.38	18.55	19.37	89.6	207	99.0
DeKalb, Ill.	4.9	23.3	1.73	.639	36.94	.613	35.43	95.9	.118	6.82	18.47	19.25	89.6	118	100
Madison, Wis.	4.4	21.3	2.10	.750	35.71	.722	34.38	96.3	.154	7.33	20.53	21.33	90.9	153	98.5
Waseca, Minn.	5.0	19.6	2.56	.905	35.35	.865	33.79	95.6	.188	7.34	20.77	21.73	87.3	207	93.5
Kanawha, Ia.	4.9	21.2	2.26	.821	36.33	.780	34.51	95.0	.155	6.86	18.88	19.87	88.7	205	98.0
Ames, Ia.	4.3	20.2	2.29	.790	34.50	.755	32.97	95.6	.177	7.16	20.76	21.85	88.8	199	99.0
Brookings, S. D.	4.0	13.8	2.52	.857	34.01	.810	32.14	94.5	.174	7.02	20.65	21.85	89.1	210	98.5
Bozeman, Mont.	6.2	25.7	2.25	.804	35.73	.755	33.56	93.9	.155	6.89	19.28	20.53	86.3	259	98.5
Fort Collins, Col.	4.9	21.7	2.45	.710	28.97	—	—	—	.185	7.55	26.06	—	88.3	222	98.0
Davis, Cal.	5.1	25.3	2.25	.876	38.93	.835	37.11	95.3	.192	8.53	21.92	22.99	86.1	202	94.0
Manchuria, average	4.9	21.6	2.23	.778	35.04	.748	33.99	95.4	.158	6.81	20.32	20.70	88.6	194	97.6

TABLE XXI—Continued

Variety and location where barley was grown	Mois- ture con- tent of malt	Kerned weight of malt, dry basis	Nitro- gen in malt wort	Soluble nitrogen in malt N	Perma- nently soluble nitrogen as % of wort N	Perma- nently soluble nitrogen as % of wort N	Formol nitro- gen in malt wort	Formol N as % of wort N	Formol N as % of wort soluble N	Recovery of malt from barley, dry basis	Dye stain power of malt	Endosperm character of malt. Index of malloivness
Trebi	%	Mg.	%	%	%	%	%	%	%	%	°L	%
East Lansing, Mich.	5.7	28.5	2.30	.640	27.83	26.26	.130	5.65	20.31	21.52	185	90.0
Urbana, Ill.	4.9	33.4	1.81	.524	28.95	27.35	.081	4.47	15.45	16.36	151	93.0
DeKalb, Ill.	4.5	34.6	1.47	.483	32.86	31.22	.084	5.71	17.39	18.30	88	98.0
Madison, Wis.	5.0	29.5	1.73	.536	30.98	29.34	.099	5.72	18.47	19.37	103	98.5
Waseca, Minn.	4.7	27.3	2.47	.758	30.69	29.03	.141	5.71	18.60	19.67	175	95.0
Kanawha, Ia.	4.6	28.7	2.19	.614	28.04	26.62	.099	4.52	16.12	16.98	163	96.5
Annes, Ia.	5.2	29.3	2.02	.585	28.96	27.77	.099	4.90	16.92	17.65	142	100
Brookings, S. D.	4.9	19.6	2.56	.784	30.63	28.98	.136	5.31	17.35	18.33	222	95.5
Bozeman, Mont.	5.2	36.2	2.38	.559	23.49	22.23	.081	3.40	14.49	15.31	189	74.0
Fort Collins, Col.	4.5	28.8	2.01	.567	28.21	26.91	.094	4.68	16.57	17.37	165	76.0
Trebi, average	4.9	29.6	2.09	.605	29.06	27.59	.104	4.98	17.17	18.09	158	91.6

yielded higher than Trebi. The data on yield of extract for the three years are given in Tables XV and XXIII.

The average protein content of barleys and malts from all stations was lower in 1936 than in either of the previous two years and averaged about the same as in 1935 for the six stations. This is somewhat contrary to expectations as in general the hot, dry weather and hastened maturity are considered likely to increase protein content. The

TABLE XXII

DIASTATIC POWER EXPRESSED IN DEGREES LINTNER OF MALTS FROM THE FIVE VARIETIES OF BARLEY GROWN IN THE REGIONAL SERIES IN 1934, 1935, AND 1936

Variety	Year	Location where barley was grown														Average ^a
		Columbus, Ohio	East Lon- sing, Mich.	Urbana, Ill.	DeKalb, Ill.	Madison, Wis.	Waseca, Minn.	Kanawha, Ia.	Emmetts- burg ² or Ames, Ia.	Brookings, S. D.	Fargo, N. D.	Lincoln, Neb.	Bozeman, Mont.	Fort Collins, Colo.	Davis, Cal.	
Oderbrucker	1934 ¹	° L.	° L.	° L.	° L.	° L.	° L.	° L.	° L.	° L.	° L.	° L.	° L.	° L.	° L.	° L.
	1935	175	142	—	—	152	184	109	—	164	167	—	167	140	—	153.1
	1936	—	145	211	—	175	172	—	212	220	140	192	205	191	138	182.1
Wisconsin Barbless	1934	—	51	—	—	107	81	57	—	73	97	—	77	—	—	77.6
	1935	139	130	152	—	124	149	—	152	151	—	117	114	129	115	133.8
	1936	—	119	116	74	89	110	121	124	104	—	—	155	129	153	117.6
Velvet	1934	—	76	—	—	135	152	—	—	94	93	—	96	91	—	105.3
	1935	177	160	164	—	158	152	—	144	215	179	148	155	167	—	165.4
	1936	—	118	150	95	123	171	165	170	160	—	—	162	175	191	152.7
Manchuria	1934	—	143	—	—	133	154	—	—	137	133	—	77	96	—	124.7
	1935	177	164	205	—	187	184	—	217	220	208	207	165	208	148	190.8
	1936	—	158	207	118	153	207 ³	205	199	210	—	—	259	222	202	194.5
Trebi	1934	—	61	—	—	78	93	71	—	70	60	—	78	87	—	75.9
	1935	184	123	127	—	131	140	—	131	180	—	135	149	135	—	143.5
	1936	—	185	151	88	103	175	163	142	222	—	—	180	165	—	158.3

¹ The malts produced in the 1934 studies were dried below 4% moisture which reduced the diastatic power below the values for the latter two years.

² Barley grown at Emmetsburg in 1935, and at Ames, Iowa, in 1936.

³ Manchuria (Minn. 124) was substituted for Manchuria (N. Dak. 2121) in the Waseca series in 1936 as the latter was not grown.

⁴ Average for all stations; see Table XXIII for comparable averages.

protein content of the barleys and malts from the stations in the north-central area was lower with the exception of the varieties grown at Waseca, Minnesota, where all the barleys except Wisconsin Barbless were higher than in 1935. Likewise three of the four varieties grown at Davis, California, were higher than in 1935. The relative order of the varieties has shifted significantly in all three years, with Trebi and Wisconsin Barbless ranking the lowest each year except 1934, when Manchuria was lower than Wisconsin Barbless. The data on protein content are given in Tables XVI, XVII and XXIII.

Variety	Year	Yield of barley, bushels per acre	Bushel of weight of barley, 4% dry basis	Bushel of weight of malt, 4% dry basis	Kernel of weight of malt, 4% dry basis	Skinned and broken kernels	Weight of hull of barley	Index ² of mellowness of endosperm of dry barley A	Index ¹ of mellowness of endosperm of steeped barley 24 to 30 hrs. B	Index of mellowness of steeped malt C	Estimated time of steeped malt to reach 46% moisture	Time steeped	Moisture content steeped barley	Moisture content of sprouted barley	Recovery of malt from dry basis	Ash content of barley	Yield of extract of malt, dry basis	Total protein in malt, N X 6.25	Total protein in wort as protein, N X 6.25	Soluble nitrogen in malt, N X 6.25	Dia-static power of malt
Oderbrucker	1934	21.6	40.3	36.5	28.2	26.4	7.0	24.7	53.4	85.6	Hrs.	Hrs.	%	%	%	%	%	%	%	%	
	1935	36.3	38.6	35.3	25.5	22.0	2.7	67.5	95.4	94.3	33.3	33.3	46.3	51.2	87.5	2.51	71.8	17.35 ³	17.59	5.79	150.5
	1936	31.6	39.4	34.2	24.7	21.9	4.6	36.8	97.6	97.9	31.4	34.5	47.1	47.1	87.6	2.83	71.1	14.75	15.16	5.56	189.7
	3-year average	29.9	39.4	35.3	26.1	23.4	4.8	43.0	92.1	92.6	33.0	34.4	46.8	48.4	86.6	2.85	72.4	15.78	15.84	5.46	180.3
Wisconsin Baraboo	1934	34.1	39.2	36.5	29.2 ³	26.5	5.4	20.5	67.4	79.2	40.3	40.8	46.1	50.7	88.4	2.42	69.7	17.18 ⁴	16.54	4.30	80.5
	1935	54.9	40.1	37.0	27.4	24.2	4.8	56.9	94.3	94.2	39.6	43.2	46.7	45.5	88.3	2.97	71.0	14.06	13.97	4.40	136.7
	1936	39.0	37.8	34.2	26.1	24.1	2.9	35.7	98.2	97.7	30.9	42.5	48.0	45.8	89.9	2.83	68.7	13.17	13.90	3.75	116.3
	3-year average	42.7	39.0	35.9	27.6	24.9	4.4	37.7	86.6	90.4	36.9	42.2	46.9	47.3	88.9	2.74	69.8	14.80	14.80	4.01	111.2
Velvet	1934	31.6	40.4	36.6	27.6 ³	25.5	5.4	23.0	57.9	81.1	34.2	36.2	46.4	50.5	88.2	2.39	71.2	17.29 ⁴	17.14	5.33	110.5
	1935	45.9	39.2	34.7	24.4	20.7	4.9	59.4	93.0	93.2	35.7	37.2	46.7	46.6	86.3	3.28	72.0	14.42	14.44	5.14	164.0
	1936	37.4	39.7	34.7	24.7	22.2	3.5	37.2	94.5	93.0	29.1	38.0	47.4	45.6	89.5	2.84	70.9	14.75	14.25	4.67	148.8
	3-year average	38.3	40.0	35.8	25.6	22.8	4.6	39.9	81.8	89.1	33.0	37.1	46.8	47.6	88.0	2.83	71.4	15.48	15.28	5.05	141.4
Manchuria	1934	29.4	40.1	36.2	26.2 ³	23.8	7.1	28.1	68.4	86.8	33.8	34.2	46.1	51.7	85.6	2.31	72.7	16.05 ⁴	16.57	5.42	127.8
	1935	27.5	39.2	34.7	24.2	21.1	4.4	68.9	97.0	95.7	31.7	30.7	46.4	46.6	84.5	3.29	73.3	15.16	14.89	5.96	189.3
	1936	30.2	39.2	33.2	24.2	20.8	6.1	38.2	93.7	97.2	26.7	34.2	48.6	47.0	88.7	2.81	72.1	14.49	14.18	5.34	195.7
	3-year average	32.4	39.5	34.7	24.9	21.9	5.9	45.1	86.4	92.6	30.4	33.0	47.0	48.4	86.3	2.80	72.7	15.23	15.21	5.57	171.9
Trebi	1934	47.9	39.0	35.2	35.4 ³	33.3	5.0	14.7	45.8	73.1	45.3	46.0	46.2	50.2	89.5	2.16	72.4	14.78 ⁴	14.75	3.96	75.2
	1935	50.8	38.8	36.0	33.2	29.5	5.6	47.7	85.5	92.1	36.1	42.5	47.1	46.2	88.0	3.00	73.1	13.31	13.24	4.10	142.3
	1936	46.3	38.4	35.2	32.0	28.3	8.5	27.7	86.6	91.6	37.4	44.2	47.4	46.3	90.5	2.67	71.0	14.06	14.19	4.05	172.8
	3-year average	48.3	38.7	34.8	33.5	30.4	6.4	30.1	72.5	85.6	39.6	44.2	46.9	47.6	89.3	2.61	72.2	14.05	14.06	4.06	130.1
Annual average 5 varieties	1934	32.9	39.8	36.2	29.3	27.1	6.0	22.2	58.6	81.2	37.5	38.5	46.2	50.9	87.8	2.36	71.6	16.64	16.52	4.90	108.9
	1935	43.1	39.3	35.9	27.0	23.5	4.5	60.1	93.0	93.5	35.4	37.4	46.8	46.4	86.4	3.15	72.7	14.32	14.24	4.96	164.4
	1936	36.9	38.9	35.9	26.0	23.5	5.1	35.1	94.1	95.5	30.9	38.7	47.7	46.4	86.2	2.80	70.8	14.26	14.26	4.37	107.7
	Minimum significant difference between 8-year varieties	3.7	0.8	1.0	0.9	1.4	—	4.8	5.7	—	—	—	—	—	1.3	0.08	1.2	0.66	0.56	0.28	13.7

¹ Five-stations average as data from Kanawha, Iowa, were not obtained in 1934.

² Index of mellowness was calculated as follows: Number of steady kernels X 0. number half-steady kernels X 1, and sums added.

³ Moisture content of barley was not determined at the same time kernel weight was obtained. Kernel weights of 1934 averages were calculated for comparative purposes on the basis of 12% moisture in the barleys.

⁴ Four barleys only averaged in 1934 barley protein averages.

The average difference in protein content between the barleys and malts is consistent although not large in the three years. The differences in protein content between the barley and malt for all five varieties averaged together are as follows: 1934, 0.23%; 1935, 0.44%; and 1936, 0.18%. Sufficient data are not available to correlate these differences with any particular group of factors in the barleys and malts.

The average soluble nitrogen in the wort was lower for the malts from Oderbrucker, Wisconsin Barbless, Velvet and Manchuria in 1936 than in the previous two years. The percentage of soluble nitrogen in the wort is seemingly as closely correlated with total nitrogen in the barley and malt as with the diastatic power of the malt. This relationship is indicated by the fact that the soluble nitrogen is consistently lower in Wisconsin Barbless and Trebi than in the other three varieties. The relative order of the five varieties with respect to soluble nitrogen in the wort remained the same as in 1935. The data are presented in Tables XVIII and XXIII.

The modification in the malts as measured by protein degradation has varied from year to year and is different for the five varieties. The summary data showing nitrogen modification on the basis of total malt nitrogen, soluble nitrogen in the laboratory wort, and the per cent of the malt nitrogen which is soluble in the wort, are presented in Table XIX. These data are compared with kernel weight of the malts, recovery of malt from barley and diastatic power of the malts as suggested in the excellent review article by Laufer (1937). Data on permanently soluble nitrogen in the worts and formol nitrogen in the worts were not obtained for the malts from the 1934 and 1935 barleys; therefore the comparisons on the nitrogen modification on the three years' data were limited to soluble nitrogen in the worts. A more detailed comparison of modification was made on the malts from the 1936 barleys and this is given in Tables XX and XXI.

The nitrogen modification based on the per cent of the malt nitrogen which is soluble in the wort shows a significant negative correlation with the percentage recovery of malt from the barley. Seasonal and varietal differences are evident, however, in the summary data presented in Table XIX. The malts from the 1934 barleys were lowest in soluble nitrogen expressed as per cent of the total nitrogen in the malt. This probably is influenced somewhat by the method of drying the malts in 1934 and is also influenced by the abnormally high malt nitrogen. The malts from the 1935 barleys are significantly higher in per cent of malt nitrogen soluble in the wort and lower in

percentage recovery than either 1934 or 1936. The varieties likewise show differences in nitrogen modification based upon the ratio of soluble nitrogen in the wort to total malt nitrogen when averages are compared within an individual year as well as for the three-year average. Manchuria ranks highest in percentage of the malt nitrogen soluble in the wort, Oderbrucker, Velvet, Trebi, and Wisconsin Barbless following in descending order.

The more complete study of nitrogen modification made on the malts from the 1936 barleys suggests that Manchuria, Oderbrucker and Velvet are essentially alike based on the average of the eleven malts for each variety. The averages for the malts from the Wisconsin Barbless variety are appreciably lower than in the above three varieties but higher than Trebi. While the varietal averages for the malts show the general differences, a study of the individual malts from the barley varieties grown at the different locations shows many exceptions to the relative varietal ranking given in the summary table. (Tables XX and XXI.) The data given in more detailed form (Table XXI) show the range of variation found in the malts within a barley variety grown at the different locations or between varieties grown at one location. A number of interesting comparisons might be made from the data presented. The writers feel however that the one year's results are of value mainly in pointing out the impossibility of using any single factor in evaluating modification in a group of malts. This is illustrated by a comparison of the malts made from the low protein barleys from DeKalb, Illinois, with those from the relatively high nitrogen barleys grown at Waseca, Minnesota, and these in turn with the malts from the barleys grown at Bozeman, Montana. The data on the study of the nitrogen fractions are presented, therefore, as a progress report to make them available rather than to draw conclusions or attempt to discuss the differences. A study of chemical and physical methods for determining modification of malts is being continued.

The diastatic power of the malts from the five varieties showed considerable variation in 1936. The average diastatic power for the Oderbrucker, Manchuria and Trebi varieties was somewhat higher than in 1935. The diastatic power for the malts of Wisconsin Barbless and Velvet averaged lower than in 1935. Malts from the Wisconsin Barbless barleys grown in the north-central area were all lower in diastatic power than in the previous year. No general trend was evident in the diastatic power of the malts from the other varieties in this same area. The malts from the barleys grown at Bozeman, Montana, Fort Collins, Colorado, and Davis, California, were higher

in diastatic power than in the previous two years. The relative order of the varieties was the same as in 1935 except that Trebi and Velvet were reversed, the former being slightly higher in 1936. The data on diastatic power of the malts are given in Tables XXII and XXIII. The season in which the barleys were grown has a marked influence on diastatic power. This contrast has been exaggerated somewhat by the relatively low diastatic powers given for 1934. The malts produced from the 1934 barleys were dried for a longer period and to a lower moisture content which resulted in comparatively low diastatic values. The varieties have shown marked differences in diastatic power in each of the three years.

The time required in the mashing process to convert the starch to dextrins, "conversion time," has been used as a comparative measure of the dextrinizing capacity of the malts. The conversion time in the mashing process has not been determined in the exact intervals of minutes, but rather as outlined in the Official Methods of the American Society of Brewing Chemists. The comparison of conversion time for the various malts has been made by taking the average time in minutes recorded for each malt, as for example: less than 5 minutes was arbitrarily assigned the value of 4 minutes; 5 to 7 minutes, 6 minutes; 10 to 15 minutes, 12.5, etc. These numerical values in minutes were used in obtaining averages and in the statistical analysis.

The average rate of conversion of the starch to dextrins varied for the five varieties grown and malted in 1936. The average time of conversion for the five varieties grown at the eleven stations, listed in the earlier tables for 1936, was as follows: Oderbrucker 4.6, Manchuria 4.9, Velvet 5.7, Wisconsin Barbless 7.6, and Trebi 10.3 minutes, respectively. The time of conversion in the 1936 malts varied slightly from those in 1935. The malts from the different varieties varied decidedly in their ranking as to conversion time in the two years. The average time of conversion for the five varieties grown at twelve locations in 1935 was as follows: Manchuria 4.1, Velvet 4.4, Oderbrucker 4.6, Wisconsin Barbless 6.6, and Trebi 9.0, respectively. The time of conversion in the malts from the five varieties grown at eight stations in 1934 was slower than in the two years following. This may have been due in part to the difference in procedure in drying the malts in 1934. The malts were dried longer and at a higher temperature in the later phases of the drying which resulted in a lower moisture content and a lower diastatic power in the malts produced in 1934. The ranking of the malts for conversion time also varied from the two previous years. The average time of conversion for the malts from

the five varieties in 1934 was as follows: Oderbrucker 5.1, Velvet 5.9, Manchuria 6.4, Wisconsin Barbless 7.9, and Trebi 12.6 minutes, respectively. The three-year average for the five varieties—Oderbrucker 4.8, Manchuria 5.1, Velvet 5.3, Wisconsin Barbless 7.4, and Trebi 10.6 minutes, respectively—suggests relatively little difference in the conversion time in the first three varieties as the minimum significant difference between varietal averages is 1.8 minutes. These three varieties have shifted in their relative ranking for conversion time in each of the three years. The malts from Wisconsin Barbless and Trebi did not change position in any year and were significantly slower in time of conversion, Trebi malts taking twice the time required for the malts from the first three varieties. The average conversion time in minutes for the malts from the five varieties grown at the six stations was 7.6, 5.9, and 6.2, in 1934, 1935, and 1936, respectively.

The conversion time of the malts from the five varieties shows a significant negative correlation with diastatic power. The correlation is significant for each of the three years and for the combined three years' data. The correlation is higher in the malts produced in 1934 under the drying conditions which resulted in a lower diastatic power than in the last two years. The correlation values for each of the three years are as follows: 1934, $\gamma = -0.620$, 1935, $\gamma = -0.381$, and 1936, $\gamma = -0.387$. For the three years' data combined, $\gamma = -.510$. The 5% and 1% levels of significance are -0.197 and -0.260 respectively for the combined three years' data. In other words, the negative correlation between conversion time and diastatic power based on the three years' data is highly significant.

The analysis of variance applied to the three years' data on conversion time shows differences in reaction and interaction from that of diastatic power (Table XXV). Varieties appear to be the most important determining factor in conversion time with years second; location where grown seems of little importance. Interactions between varieties, stations and years are low and do not suggest differential responses in time of conversion.

These comparisons for the three years are only relative because the time of conversion was not recorded at close enough intervals to show the finer differences between Oderbrucker, Manchuria and Velvet. The results suggest, however, the importance of applying more precise methods of distinguishing between the different diastatic enzymes in the study of malts from the different barley varieties. The 1934 values also substantiate the idea that the dextrinizing enzyme or alpha-amylase is more heat stable than the saccharifying enzyme or beta-amylase.

Summary of Three Years' Data

As stated earlier in the paper, three years' data covering widely different conditions and a very limited number of varieties are not sufficient to warrant conclusive statements. The results are encouraging, however, in that they show consistent differences in response between the varieties used in the investigations, between the different stations where the five varieties are grown and between the three seasons. In other words, from the standpoint of methods, the investigational procedure and factors used to measure differences in quality appear to be applicable to the problem and fairly reliable. The results are further encouraging in that the varieties performed in a rather constant manner at the different stations and in the different years. Such information, if reliably founded on the limited scope of the present data, is encouraging for it suggests a relative stability of the varieties and the importance of germplasm in so far as quality has been measured by the factors used.

The average of the three years' data as given in each table for all stations for each of the five varieties is somewhat misleading as they tend to level out the varietal and seasonal differences in the various factors measured. The average of the data for the three years for each of the five varieties grown at the six comparable stations gives a more reliable comparison of the seasonal effect on quality (Table XXIII).

Averages for the stations within the given barley areas would perhaps more nearly reflect the condition of the general run of the commercial barley for each year. This phase of the problem of barley quality is not a part of the present paper and is mentioned merely to guard against the drawing of conclusions and applying them to the commercial crop of barley.

The average diastatic power of the malts produced in 1934 was very low and conversion time somewhat longer in contrast to those of 1935 and 1936. This is due in large part to the methods used in malting the 1934 barleys. The malts were dried to a final moisture content of about 3.6% in 1934. Later studies on the influence of drying showed that the low moistures reached in the malts in 1934 reduced the diastatic power below the values which would be obtained with a final moisture content of 4 to 5%. Therefore, the low figures for diastatic power in 1934 and somewhat slower rate of conversion are caused probably by prolonged drying rather than by seasonal conditions. In general the diastatic power should have been high in 1934 as there is a

relatively high positive correlation between protein and diastatic power.

Two characteristics of the 1936 barleys and malts are shown in the average data presented in Table XXIII, namely, the relatively low protein content of the 1936 barleys and the correspondingly low soluble nitrogen in the wort. This is somewhat contrary to expectations as a hot, dry season and hastened maturity are said to increase the protein content of barley. There is need of experimental work on the physiology of the barley plant as illustrated here and in numerous other instances.

A brief statistical analysis of yield and the more important quality factors was made. An analysis of variance was used for the data from the six stations where all five varieties were grown during the three-year period and the results are presented in Tables XXIV and XXV. Since only three years and a limited number of varieties were used, this analysis is presented as a preliminary indication of the relationships which may exist for the factors studied. The analysis was made on yield and on each of the quality factors as affected by varieties, stations, and years; the first order interactions, varieties $\times$ stations, varieties $\times$ years, and stations $\times$ years; and the second order interaction, varieties $\times$ stations $\times$ years. The following factors were studied: yield of grain, bushel weight of barley, bushel weight of malt, kernel weight of barley, kernel weight of malt, weight of hulls of barley, estimated time steeped to reach 46% moisture, mellowness of endosperm of dry barley, mellowness of endosperm after 24 to 30 hours' steep and drying, recovery of malt from barley, ash in barley, extract in malt, protein in barley, protein in malt, soluble nitrogen in wort as protein, diastatic power of malt and conversion time in mashing. The "F values" were calculated for each of these factors. These values represent the ratio of the second order interaction variance (varieties $\times$ stations $\times$ years) to the variance due to varieties, stations, years, etc. At the end of each table are given the 5% and 1% "F values" appropriate to each comparison. The design of the experiment was such that the second order interaction variance was the most appropriate to use as error. A significant "F value" for varieties indicates that some real differences exist among the varieties. The same is true for stations and years. Significant "F values" for first order interactions indicate that differential response is greater than that which might be attributed to chance alone.

The values of minimum significant differences for each factor were also obtained. These are appropriate to use in comparing the differ-

TABLE XXIV

ANALYSIS OF VARIANCE OF YIELD, BUSHEL WEIGHT OF BARLEY AND MALT, KERNEL WEIGHT OF BARLEY AND MALT, WEIGHT OF HULL OF BARLEY, TIME STEEPED TO REACH 46% MOISTURE, MELLOWNESS OF ENDOSPERM BEFORE AND AFTER STEEPING, AND RECOVERY OF MALT FROM BARLEY FOR FIVE VARIETIES OF BARLEY,¹ SIX STATIONS,¹ AND THREE YEARS.^{1, 2}

Variation due to	De- grees of free- dom	Yield of barley bu. per A.		Bushel weight of barley, dry basis		Bushel weight of malt, 4% basis		Kernel weight of barley, dry basis		Kernel weight of malt, dry basis		Weight of hull of bar- ley, dry basis		Estimated time to reach 46% moisture		Mellowness of endosperm, dry barley		Mellowness of endosperm after steeping 24-30 hrs. ^a		Recovery of malt from barley, dry basis		1% F. point value	
		Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value
Varieties	4	1,018.36	33.71	4.55	2.94	4.89	2.49	226.42	133.19	202.50	45.56	4.63	4.51	239.99	6.43	608.79	11.74	481.09	7.79	32.80	9.11	2.61	3.83
Stations	5	5,517.08	182.02	147.85	95.39	143.64	73.29	217.15	127.73	208.12	46.83	33.30	32.50	878.16	23.54	736.78	14.21	194.02	3.16	20.39	5.06	2.45	3.51
Years	2	1,149.17	38.04	6.04	3.90	38.30	19.54	102.97	60.57	131.05	29.49	41.59	40.48	341.10	9.14	11,195.07	214.80	10,215.62	165.43	62.22	17.28	3.23	5.18
Varieties × stations	20	109.22	3.62	1.91	1.23	2.16	1.10	1.59	0.93	3.48	0.78	1.55	1.50	44.56	1.19	58.04	1.12	37.27	0.60	3.18	0.88	1.85	2.40
Varieties × years	8	89.40	2.96	2.27	1.46	1.48	0.76	1.31	0.77	3.40	0.77	2.42	2.35	41.77	1.12	46.89	0.90	90.81	1.47	3.05	0.85	2.18	2.99
Stations × years	10	653.43	21.63	52.65	33.97	32.84	16.76	57.80	34.00	43.12	9.70	11.30	11.00	304.18	8.15	571.33	11.02	179.63	2.83	27.69	7.69	2.09	2.82
Varieties × stations × years	40	30.21		1.55				1.70		4.44		1.03		37.30		51.84		61.75		3.60			
Total	89																						

¹ Stations used are: East Lansing, Mich., Madison, Wis., Waseca, Minn., Kanawha, Iowa (data from Emmetsburg, Iowa, were used in 1935 instead of Kanawha as samples were not malted from the latter station), Brookings, S. D., and Bozeman, Montana. Varieties used are: Odeurucker, Wisconsin Barless, Velvet, Manchuria, and Trebil. Years represent barleys grown in 1934, 1935, and 1936.

² Manchuria (Minn. 185) at Waseca, Minnesota, was substituted in 1936 for Manchuria (N. Dak. 2121).

³ Five stations only were used in analysis of variance of bushel weight of malt and mellowness of endosperm after steeping, the data from Kanawha, Iowa, not being available. Degrees of freedom are reduced accordingly for these two factors and F. values for the 5% and 1% points are changed as follows: 5% point—2.67, 2.67, 2.67, 2.67, 2.67; 1% point—3.91, 3.91, 3.91, 3.91, 3.91.

TABLE XXV

ANALYSIS OF VARIANCE OF ASH IN BARLEY, EXTRACT IN MALT, PROTEIN IN BARLEY AND MALT, SOLUBLE NITROGEN AS PROTEIN IN WORT FROM MALT, AND DIASTATIC POWER OF MALT FOR FIVE VARIETIES OF BARLEY,¹ SIX STATIONS,¹ AND THREE YEARS^{1, 2}

Variation due to	De- grees of freedom	Ash in barley		Extract in malt, dry basis		Protein in barley ³		Protein in malt		Soluble N. in wort as protein		Diastatic power of malt		Conversion time in mashing		5% point		1% point	
		Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value	Vari- ance	F. value
Varieties	4	.172	11.90	24.24	7.91	8.78	9.03	7.84	10.90	10.14	57.74	14,938.93	35.44	74.34	9.72	2.61	2.45	3.83	3.51
Stations	5	.458	31.59	40.97	13.38	5.78	5.95	11.35	15.77	4.97	28.28	2,393.18	5.66	5.93	0.77	2.45	2.45	3.51	3.51
Years	2	4.660	321.38	32.34	10.56	52.53	54.07	49.20	68.36	1.55	8.82	32,722.21	77.39	24.30	3.18	3.23	3.23	5.18	5.18
Varieties × stations	20	1.800	124.14	1.17	0.38	0.80	0.82	0.66	0.91	0.20	1.16	456.90	1.08	6.84	0.89	1.85	1.85	2.40	2.40
Varieties × years	8	.050	3.45	0.62	0.20	2.26	2.33	1.61	2.24	0.31	1.78	1,194.71	2.82	4.43	0.58	2.18	2.18	2.99	2.99
Stations × years	10	.186	12.83	24.15	7.89	10.05	10.35	13.13	18.24	1.95	11.10	2,286.58	5.41	15.22	1.99	2.09	2.09	2.82	2.82
Varieties × stations × years	40	.014		3.06		0.97		0.72		0.18		422.83		7.65					
Total	89																		

¹ Stations used are: East Lansing, Mich., Madison, Wis., Waseca, Minn., Kanawha, Iowa (data from Emmetsburg, Iowa, were used in 1935 instead of Kanawha as samples were not malted from the latter station), Brookings, S. Dak., and Bozeman, Mont. Varieties used are: Oderbrucker, Wisconsin Barless, Velvet, Manchuria, and Trebi. Years represent barleys grown in 1934, 1935, and 1936.

² Manchuria (Minn. 184), at Waseca, Minnesota, was substituted in 1936 for Manchuria (N. Dak. 212) as grain from the latter was not furnished from this station in 1936.

³ Protein in malt was substituted for protein in barley in 1934 for samples from East Lansing, Mich., and Waseca, Minn.

ences between varietal means for the three-year period (Table XXIII).

By using the second order interaction (varieties $\times$ stations $\times$ years) as error the following conclusions suggest themselves.

For each of the factors studied some varieties were found to be superior to others except in the case of bushel weight of malt. This does not mean that a specific variety is superior for all of the factors, but rather that for each factor there can be shown to be a variety or varieties which are superior to others. For example it was found that for the three-year period the malt recovery of Wisconsin Barbless was significantly greater than for Oderbrucker, while for extract yield, diastatic power and conversion time the same two varieties also showed a significant difference but in the reverse order.

For each of the factors studied, except conversion, some stations were shown to differ significantly from others, *e.g.*, the kernel weight of barley at Bozeman, Montana, was found to be greater than at Brookings, South Dakota.

For each of the quality factors studied it was found that the yearly crops differed very significantly from each other.

The stations $\times$ years interaction was found to be significantly greater than error for each of the factors studied except conversion. The large interaction in every case was due in part to the radically different seasonal conditions which prevailed during the three years of the study.

The varieties $\times$ stations interaction was found to be insignificant for all of the factors studied except yield of grain, and ash in barley. In those cases where low interactions were obtained it shows that in general the varieties held the same relative rank at the different stations. They permit the extension of the results obtained in the form of regional recommendations. This may be illustrated in the case of extract of malt. In Table XV the average extract of malt of Wisconsin Barbless for the six stations during the three years is found to be 69.8%. This is the lowest value for the five varieties listed. Now since the varieties $\times$ stations interaction is not significant it is to be expected that Wisconsin Barbless will be relatively low in extract at the several stations. A detailed inspection of Table XV bears out this generalization.

The low interaction of varieties $\times$ stations suggests that the varieties produced by the barley breeder have a general regional application for all factors except yield of grain and ash in the barley. For these factors specific attention will have to be given to more localized areas.

To the barley buyer it means that he can buy a single variety over a wide geographical area and have considerable assurance that its quality will hold a relative position to that of another variety grown in the same area. This does not mean there will be no fluctuations in the quality of the variety bought. It does mean that the quality will be about the same relative to other varieties, *e.g.*, Oderbrucker will almost always give a higher diastatic power than Wisconsin Barbless no matter in what part of the area the two varieties are grown.

The varieties $\times$ years interaction was found to be insignificant for some of the factors studied and for those the varieties behaved similarly in the different years. Where the interaction attained a significant level the varieties did not react similarly in the different years. The factors where this is the case are as follows: yield of grain, per cent of hull, ash in barley, protein in barley, protein in malt, and diastatic power. The interaction for the diastatic power was probably somewhat inflated by the fact that after the first year the malting procedure was changed in an important step affecting this factor.

It appears that the relative ranking of varieties for some of the factors is more easily upset by the season than by the location. For other factors neither of these seems to play a part. And lastly there are factors in which both year and location seem to operate.

Summary

Data on the variability of malting controls for the three years are presented and discussed. Although there was considerable variability in the malting process the results are sufficiently accurate to calculate differences between varieties, stations and seasons.

The discussion of the experimental malting of the barleys grown in 1936 and the three years' data for the five varieties grown at the co-operating agricultural experiment stations are presented.

The 1936 barleys were of poorer quality than those of 1935 especially from the stations in the upper Mississippi Valley section.

The five varieties studied retained approximately the same relative positions in 1936 as in 1935, with the exception of Wisconsin Barbless, which was most adversely affected by the hot dry season which hastened maturity.

Three years' data on the five varieties are summarized and discussed. A brief statistical analysis of yield and the more important quality factors is given.

The data indicate that the varieties performed in a rather constant manner at the different stations and in the different years.

Literature Cited

- Dickson, J. G., Shands, H. L., Dickson, A. D., and Burkhart, B. A.
1935 Barley and malt studies: I. Developing new varieties of barley for malting and their properties. *Cereal Chem.* 12: 596-609.
1937 Barley and malt studies: II. Experimental malting of barleys grown in 1935. *Cereal Chem.* 14: 316-327.
- Laufer, Stephen
1937 Evaluation of malt for brewing. *Cereal Chem.* 14: 220-232.
- Shands, H. L.
1937 Barley and malt studies: III. The determination of kernel weight. *Cereal Chem.* 14: 532-539.

IMPROVING THE NUTRITIVE VALUE OF BREAD BY THE ADDITION OF DRY MILK SOLIDS¹

B. W. FAIRBANKS

Division of Animal Nutrition, University of Illinois, Urbana, Illinois

(Received for publication October 11, 1937)

It has been fifteen years since Sherman and Hawley (1922) published their widely quoted paper giving the first experimental and scientific evidence to support the recommendation of one quart of milk a day for every child. Today a study of the milk consumption figures reveals that on the average the children of the United States are consuming much less than a quart of milk per child per day. In order to appreciate fully the situation as it really exists it must be noted that this low consumption of milk is a fact in spite of the whole-hearted support given to milk consumption by the medical and dental professions, the dietitians, health organizations, government agencies, parent-teachers associations and the financial assistance provided by the dairy interests largely through their various organizations. This is neither the time nor place to speculate upon causes or attempt to formulate reasons. The fact is the point of interest.

If the recommendation of Sherman and Hawley is still nutritionally sound, the problem confronting the nutritionist today is that of increasing the consumption of milk solids by children. Any innovation must not in any way detract from the efforts made to increase the consumption of liquid milk for this has the general endorsement of all interested in child nutrition. We seek a way by which liquid milk consumption will be augmented, and it is suggested that this may be accomplished by the judicious addition of milk solids to some of the regular and staple articles found in the American diet.

There is no other article of food that is so generally and universally consumed as bread. The baking technologist has demonstrated that the addition of milk solids to the bread formula is both feasible and practicable. In fact, there is quite a list of advantages attributed to the adding of milk solids to bread. If it can be demonstrated that the addition of milk solids will increase the nutritive value of the bread, the nutritionist would be justified in accepting milk-solids bread as a means of augmenting our present inadequate liquid milk consumption. It may be argued that the above reasoning implies that bread without

¹ This investigation was made possible by the donation of funds to the University of Illinois by the American Dry Milk Institute, Inc.

the addition of milk solids is without nutritive value. No such implication is intended nor would it be justified as any student of foods would attest. There should be no objection in attempting to improve a product especially when such an improvement is a means to attain a desired objective

It is the purpose of this present work to compare the over-all nutritive value of bread made without milk solids, with bread made with the addition of 6% milk solids (based upon the weight of flour) and with bread made with the addition of 12% milk solids. The experimental technique employed in this initial work is not capable of explaining in terms of nutrition why observed differences occur. At present we are only interested in the differences, and further experiments which are better controlled and more refined in procedures will be conducted to explain that which has been observed and recorded here. While the general results of this comparison are quite predictable, it seems advisable to report the findings as the nutrition literature of today does not seem to include any observations upon this particular point.

Review of Literature

The place of wheat in the American diet and the value of milk as a supplement to wheat have been discussed in a general way by Taylor (1929). "Nutritionally considered, the place of wheat in the diet may be anywhere up to, let us say, two-thirds of the calories of the diet. The higher the proportion of wheat, or other cereal, in the diet, the greater the care necessary to secure the indispensable elements in order to avoid deficiency diseases. . . . A broad survey of modern knowledge of nutrition, in a country with the agricultural characteristics of the United States, indicates that nutritional security in the diet is to be sought in the milk supply and that wheat (and other cereals) ought to serve as fuel food."

The nutrition literature records a few attempts to feed white bread as the sole diet or as a major portion of the diet to experimental subjects. The results are not in perfect agreement probably due to variations in bread formulae or lack of uniformity in experimental procedures. Hartwell (1924) fed a diet of white bread, butter, and a salt mixture to young growing rats and reports a slow rate of growth. In gestation and lactation tests, the litters were of poor weight at birth and only a few of the young were raised to the weaning age. In two trials, Abelin (1919, 1931) fed white bread to rats. There were a marked retardation of growth, changes in the bone, infections of the skin and mucous membranes, falling of the hair, eye injuries, and general weakness. In one experiment the animals died usually after three or four months. Cosla and Vasilco (1932) fed a dog exclusively

on white bread and nutritive troubles identical with those observed in general avitaminosis and avitaminosis *B* as well as experimental scurvy were noted. Eyerly, Oclassen, and Killian (1935) fed diets composed largely of bread to rats and demonstrated no deficiencies in growth-promoting factors but excessive gains in weight were not obtained. Throughout several generations of feeding, a large amount of bread did not produce any inhibition of calcification in the young nor any decalcification in mature animals.

There is evidence that the proteins of wheat may be deficient in their nutritive value. Mitchell and Smuts (1932) have demonstrated by the paired-feeding method that wheat is deficient in the indispensable amino acid lysine, and that when lysine is added there is a large increase in the growth-promoting value of the wheat. French and Mattill (1935) conclude that the proteins of bread may be grouped in the same class as meat proteins and are inferior only to the proteins of milk and eggs. This conclusion hardly seems justified as the white bread contained milk and the supplementary effect of milk and wheat proteins is ignored.

There does seem to be agreement among investigators that the vitamin B content (B-complex) of bread is low. Its importance in practical nutrition is another matter. Eijkmann and Hulshoff Pol (1918) produced polyneuritis in fowls fed white bread. Gault (1923) noted variations in the nutritive values of patent flour breads made with different amounts of yeast cakes. Veselkin, Yaroslavtzeva, Seliber, and Bovshilk (1927) concluded that white bread containing 0.5% of baker's yeast produced about one-half the vitamin necessary to maintain weight in the pigeon. Morgan and Frederick (1935) found practically no loss of the vitamin during baking at temperatures ranging from 300° to 446° F. The addition of 4% dry milk solids did not change the vitamin B₁ content of white bread, and bread sold as "milk bread" was equal to ordinary white bread in vitamin B₁ content.

As this paper is concerned primarily with the supplementary effect of milk solids when added to a white bread formula, the following reports may be considered as having a direct bearing upon the investigation. McCollum, Simmonds, and Parsons (1921) found milk to be an effective supplement to wheat, with respect to protein, calcium, and vitamin A. Fairbanks and Mitchell (1935) report a biological value of 89.8 for the proteins of raw liquid skim milk, 88.8 for a choice commercial roller process skim milk powder, and 87.9 for a preheated spray process powder. Rose, MacLeod, and Bisbey (1923) working with human subjects report a protein storage in 12 days of 30.37 grams on bread and milk as compared to 41.44 grams on milk, 4.33 grams on

meat and 2.12 grams on soybeans. Bernfeld and Schilf (1930) fed young rats bread and water or bread and liquid milk. On diets of bread and water the rats died after about six weeks. If milk was given one to two weeks before death was expected, the animals suddenly recovered and began to gain in weight.

Materials and Methods

The three types of bread were made in the baking laboratory of the Division of Agricultural Biochemistry, Department of Agriculture, University of Minnesota, by Olof Stamberg, Fellow, American Dry Milk Institute.

Three doughs were made by the straight dough method. The flour used was a blend of spring wheat flours and typical of the ordinary commercial blends used for bread baking. The formula employed was exactly the same except for the variation in the percentage of dry milk solid with a resulting variation in absorption. The formula used was as follows, in percentages based on the amount of flour used:

3% Sugar
1% Diastatic malt extract
2% Shortening
2% Salt
3% Yeast

0, 6, or 12% Dry milk solids

The fermentation time used was two hours with about 35 minutes of pan proof. The dough was baked in one-pound bread pans at 200 to 230° C. for approximately 45 minutes and stored overnight in a proofing cabinet.

The top, bottom and side crusts were shaved off and eliminated, and the resulting bread crumb was cut into thin slices and placed for about 12 to 14 hours in a drying cabinet maintained at 40 to 50° C. The dry crumb was then ground in a meat chopper.

The dry bread crumb was shipped via express to the Division of Animal Nutrition, University of Illinois. When received it was stored under refrigeration until fed.

The experimental subjects were Albino rats weighing approximately 50 grams at the beginning of the experiment. Twenty-one rats were used and they were divided into three groups. The attempt was made to equate the three groups in respect to initial weight, sex, and breeding as determined by litter. The distribution is presented in Table 1. Each rat was confined to an individual cage.

Group 1 was fed a sole diet of water bread (0% milk solids) and water; Group 2 received 6% milk-solids bread, and water, and Group 3

received 12% milk-solids bread, and water. The feed was weighed out to the rats daily. Scattering was reduced by adding water to the bread crumb, but this was not entirely avoided, especially when large quantities of bread were fed. The amount of scattering was not enough to vitiate the results in this type of experiment. Daily weights of the rats were recorded. The experimental feeding period was 77 days.

The *ad libitum* method of feeding was employed. It is appreciated that this method of feeding has certain objectionable features in a nutrition experiment. When food intake is not controlled and equated, it is difficult to assess differences to nutritive value or to the variations in food consumed. But in this initial experiment the gross or overall nutritive value was the point of interest and this would include palatability.

TABLE I

WATER BREAD VS. 6% MILK-SOLIDS BREAD VS. 12% MILK-SOLIDS BREAD
(*Ad Libitum* Feeding)

INITIAL WEIGHT, SEX, AND LITTER OF 21 RATS DISTRIBUTED IN THREE GROUPS

Water bread				6% Milk-solids bread				12% Milk-solids bread			
Rat number	Initial weight	Sex	Litter	Rat number	Initial weight	Sex	Litter	Rat number	Initial weight	Sex	Litter
22	63	Male	L1R1	23	52	Female	L1R1	24	51	Male	L1R1
25	45	Female	L2	26	53	Male	L2	27	56	Male	L1
28	49	Female	R2	29	51	Female	R2	30	51	Male	R2
31	42	Male	R1	32	60	Male	R1	33	46	Female	R1
34	49	Female	L2R1	35	46	Female	NM	36	45	Female	NM
37	45	Female	L2R1	38	50	Male	NM	39	59	Female	L1
40	56	Male	L2	41	47	Female	R1	42	48	Male	NM

Experimental Results

The weekly food consumption and weekly gains of the individual rats are recorded by groups in Table II. The average weekly gains of the rats receiving 6% milk-solids bread were higher for every week than those receiving the water bread. The weekly gains of the 12% milk-solids bread group exceeded the average weekly gains of those receiving 6% milk-solids bread except in two instances, the ninth and tenth weeks.

Using food consumption as an index to palatability a very interesting and regular set of data is presented in Table II. The average food consumption of the rats is always the lowest in the water bread group; the 6% milk-solids group takes the middle position, while the highest average weekly food consumption is always the highest in the group receiving 12% milk-solids bread. The only exception to this statement is in the first week, when the rats receiving 6% milk-solids

TABLE II
WEEKLY FOOD CONSUMPTION AND WEEKLY GAINS OF INDIVIDUAL RATS RECORDED BY GROUPS

Water bread

Rat No.	1st Week		2nd Week		3rd Week		4th Week		5th Week		6th Week		7th Week		8th Week		9th Week		10th Week		11th Week	
	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food
22	0	42	8	76	4	119	10	105	-10	25	7	56	7	51	6	91	-5	65	-4	48	0	49
23	3	36	6	46	1	47	2	24	-3	27	-2	25	-1	20	6	6	-3	28	-2	20	9	39
28	7	40	6	62	0	51	3	46	3	35	-6	30	8	31	-8	23	0	36	2	46	-9	15
31	3	24	9	40	0	32	3	24	1	34	3	32	15	45	-2	28	-1	32	4	42	7	49
37	18	42	12	79	3	92	1	60	-3	28	3	47	Died									
40	4	42	2	55	-2	44	6	45	6	43	3	50	6	42	4	40	0	47	-14	15	13	60
Av.	5.0	38.3	7.0	62.0	1.1	66.0	5.6	55.0	-1.0	31.9	2.0	40.0	8.2	42.5	1.5	44.5	0.5	43.5	-2.8	37.5	6.3	47.0

6% Milk-solids bread

Rat No.	1st Week		2nd Week		3rd Week		4th Week		5th Week		6th Week		7th Week		8th Week		9th Week		10th Week		11th Week	
	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food
23	11	42	26	91	17	98	9	75	6	61	8	77	11	66	14	79	0	74	12	89	2	88
26	10	42	24	82	14	74	4	57	6	49	12	56	27	84	12	91	14	78	3	84	9	91
29	8	42	14	55	13	89	30	96	9	64	20	83	12	66	24	97	21	78	18	96	11	91
32	10	42	19	91	12	77	4	42	-15	64	13	68	12	66	24	97	21	113	18	114	14	91
35	12	42	14	64	3	56	12	54	-1	31	4	47	-6	21	17	55	22	73	-5	51	10	59
38	12	42	15	91	7	95	-9	30	15	61	-24	11	12	35	16	48	20	64	12	55	22	78
41	11	42	11	46	6	44	9	42	-21	10	-9	15	14	45	16	49	6	56	19	63	14	67
Av.	9.3	42.0	17.6	73.3	10.3	76.1	8.4	56.6	-4.1	48.6	3.4	51.0	10.7	54.7	15.6	72.9	11.6	78.6	9.0	80.3	10.3	77.6

12% Milk-solids bread

Rat No.	1st Week		2nd Week		3rd Week		4th Week		5th Week		6th Week		7th Week		8th Week		9th Week		10th Week		11th Week	
	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food
24	13	42	31	88	44	119	15	102	11	73	28	107	22	105	-1	79	17	98	7	90	27	109
27	13	42	25	73	31	95	-12	42	-5	21	28	107	37	98	13	78	5	73	-12	53	27	109
30	17	42	29	88	18	72	42	102	30	103	28	85	37	98	13	78	5	73	-12	53	27	109
33	14	42	22	70	24	86	-1	74	14	61	10	59	28	93	18	105	4	88	23	110	5	87
36	10	42	22	75	22	82	16	78	-7	39	17	70	28	80	18	105	-1	53	11	72	-1	52
39	11	42	24	88	30	95	4	72	16	82	-20	63	16	63	27	69	15	79	-10	52	19	81
42	11	36	20	68	11	75	10	46	6	44	24	62	32	94	10	85	7	71	13	87	11	79
Av.	12.7	41.0	26.1	78.6	25.7	89.1	10.6	69.4	9.3	60.4	12.9	69.4	20.4	80.7	16.0	88.6	7.4	81.0	7.7	85.0	15.1	88.8

bread averaged 42 grams, while those receiving 12% milk-solids bread consumed on the average 41 grams. This difference of one gram, especially during the first week, can well be ignored.

Only one rat died during the experiment and this was Rat 31 in the water bread group. The daily notes of the experiments show that this rat did very poorly at the beginning of the test, improved during the second week, after which it went off feed. There were no pronounced symptoms of any disorders. The rat was thin and in poor condition, and did show a thinness of hair coat, but this latter condition was observed in several rats receiving only water bread.

Table III summarizes the results for the individual rats by groups,

TABLE III

SUMMARY BY INDIVIDUAL RATS AND GROUPS OF RATS SHOWING GAINS, FOOD CONSUMPTION AND ECONOMY OF GAIN

Water bread							
Rat No.	Initial weight	Final weight	Gain weight	Av. daily gain	Total food consumed	Av. daily food consumed	Feed fed per gram gain
22	63	86	23	0.30	727	9.4	31.6
25	45	61	16	0.21	366	4.8	22.9
28	49	55	6	0.08	415	5.4	69.2
31	42	81	39	0.51	382	5.0	9.8
34	49	Died					
37	45	97	52	0.68	501	6.5	9.6
40	56	132	76	0.99	610	7.9	8.0
Av.	49.9	85.3	35.3	0.46	500.2	6.5	25.2
6% Milk-solids bread							
23	52	168	116	1.51	840	10.9	7.2
26	53	186	133	1.73	808	10.5	6.1
29	51	196	145	1.88	847	11.0	5.8
32	60	202	142	1.84	844	11.0	5.9
35	46	119	73	0.95	550	7.1	7.5
38	50	138	88	1.14	620	8.1	7.0
41	47	121	74	0.96	479	6.2	6.5
Av.	51.3	161.4	110.1	1.43	712.6	9.3	6.6
12% Milk-solids bread							
24	51	265	214	2.78	1012	13.1	4.7
27	56	194	138	1.79	729	9.5	5.3
30	51	261	210	2.73	947	12.3	4.5
33	46	157	111	1.44	664	8.6	6.0
36	45	188	143	1.86	752	9.8	5.3
39	59	206	147	1.91	796	10.3	5.4
42	48	233	185	2.40	926	12.0	5.0
Av.	50.9	214.9	164.0	2.13	832.3	10.8	5.2

while Table IV is a summary of the results by groups. The rats receiving water bread gained 35.3 grams, those of the second group 110.1 grams, while the rats on 12% milk-solids bread gained on the average 164.0 grams. The gains by groups are presented graphically in Figure 1.

TABLE IV
SUMMARY OF RESULTS BY GROUPS

Group	Av. initial weight	Av. final weight	Av. gain	Av. daily gain	Av. total food consumed	Av. daily food consumed	Av. feed fed per gram of gain
Water bread	49.9	85.3	35.3	0.46	500.2	6.5	25.2
6% Milk-solids bread	51.3	161.4	110.1	1.43	712.6	9.3	6.6
12% Milk-solids bread	50.9	214.9	164.0	2.13	832.3	10.8	5.2

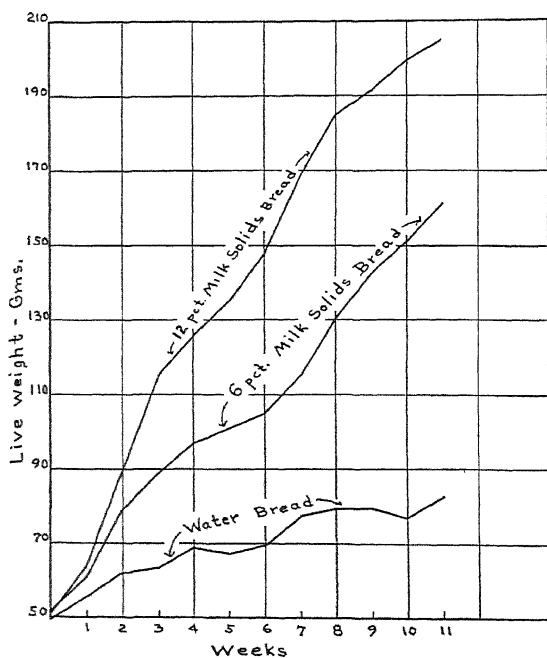


Figure 1. Growth curves of rats fed water bread, 6% milk-solids bread, and 12% milk-solids bread (*ad libitum* feeding).

The total food consumed by the rats in the water bread group was 500.2 grams per rat, for the second group 712.6 grams, and for the third group 832.3 grams. The weakness of *ad libitum* method of feeding is here revealed. All of the observed differences in gains can not be attributed to differences in nutritive values of the three types of

bread, as food consumption in the three groups is not the same. The influence of differences in palatability has not been ruled out. But it is a safe assumption, after food consumption figures are compared to gains, that not all of the additional gains of the second and third groups as compared to the water bread group are due alone to a greater food consumption. This assumption is substantiated in the figures under

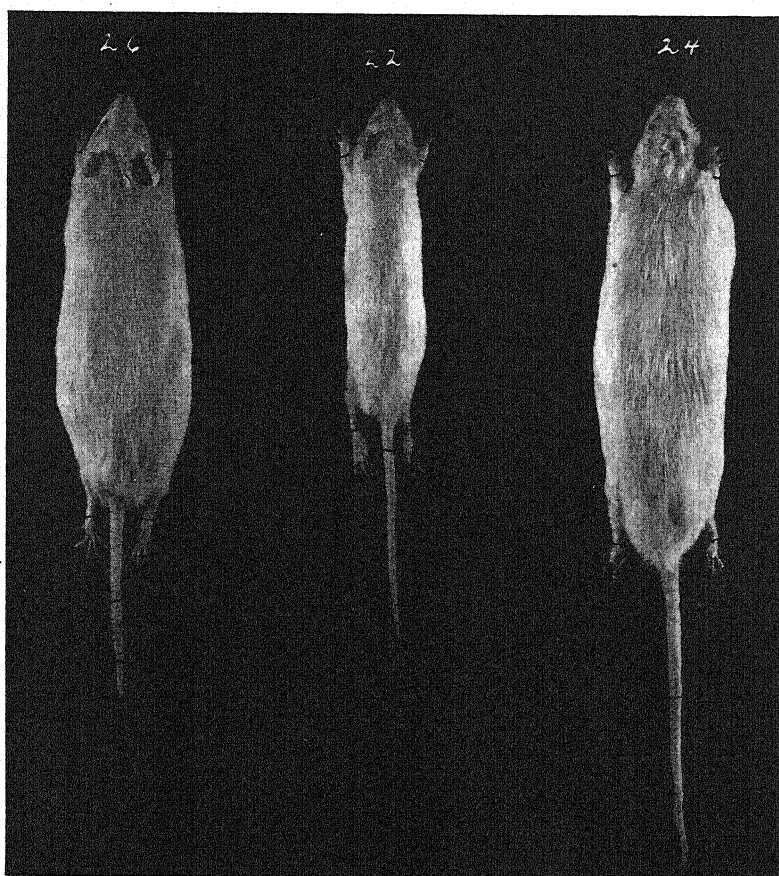


Figure 2. Skin mounting of representative rat from each group.

the average feed fed per gram of gain. It is admitted that these figures can not be taken as a true index of the comparative nutritive values of the three types of bread, as differences in maintenance requirements are not considered. But it can be said that 5.2 grams of 12% milk-solids bread satisfied the maintenance requirements of a much heavier rat than the other two groups and put on a gram of gain,

while in the case of the 6% milk-solids bread, 6.6 grams of bread maintained a smaller rat than the third group and a much larger rat than the first group and put on a gram of gain; and it required 25.2 grams of water bread to maintain a much smaller rat and put on an equal unit of gain. It therefore seems reasonable to assume that differences in nutritive values of the three types have been qualita-

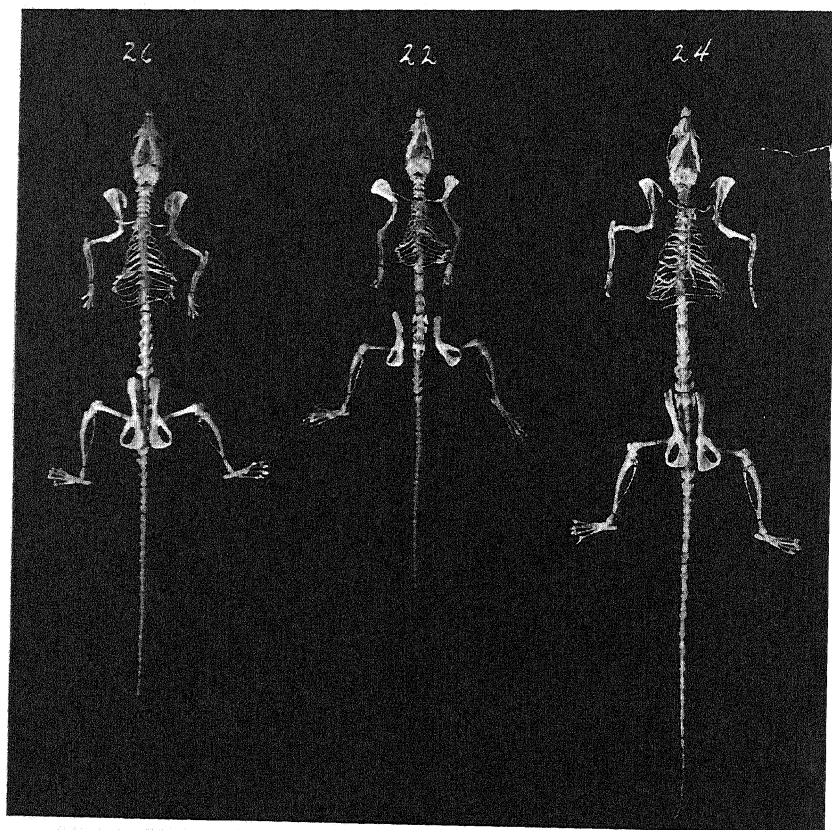


Figure 3. Skeleton mounting of rat from each group.

tively demonstrated but not quantitatively. From a practical point of view perhaps these fundamental points of interpretation of experimental data are not significant but they have been included for the sake of scientific accuracy and completeness.

L. A. Adams, Department of Zoology of the University of Illinois, made a skeleton and skin mounting of a representative rat from each of the three groups. (Figures 2 and 3). Rat 22 received water bread, Rat 26 received 6% milk-solids bread, and Rat 24 was fed the 12%

milk-solids bread. Dr. Adams reports that he experienced great difficulty in mounting the skeleton of Rat 22, as the bones were very fragile and had a tendency to crumble and break with handling.

Conclusions

By the *ad libitum* method of feeding it has been demonstrated that the addition of milk solids to a water bread (no milk) formula increases the nutritive value of the bread.

There is evidence that the nutritive value of bread containing 12% milk solids is of a higher order than bread containing 6% milk solids.

Most nutritionists are in agreement that the present consumption of liquid milk should be maintained and that efforts should be continued to increase it. Statistics indicate that the per capita consumption of liquid milk is below that recommended by authorities in nutrition. The results of this experiment indicate that the addition of milk solids to bread is an excellent method of augmenting milk consumption and thereby improving the dietary of the American people.

Literature Cited

- Abelin, I.
1919 Bread questions. *Biochem. Z.* 215: 162-190.
1931 The bread question. III. On the physiological effects of whole grain bread. *Ibid.* 232: 278-294.
- Bernfeld, A., and Schilf, E.
1930 Vitamin content of bakery products made with baking powder and with yeast. Also a contribution to the comparison of yeast extracts active toward blood vessels and the intestine with the vitamins of yeast. *Biochem. Z.* 224: 434-436.
- Cosla, O. Kaufmann, and Vasilco, O.
1932 Experimental studies on the role of white bread in nutrition. *Arch. mal. dig. nutr.* 22: 261.
- Eijkman, C., and Hulshoff Pol, D. J.
1918 Experiments on the nutritive value of standard brown bread and white bread. *Proc. Acad. Sci. Amsterdam* 21: 48-52.
- Eyerly, K., Oclassen, C., and Killian, J. A.
1935 Observations on the nutritional value of bread in the diet of human subjects and experimental animals. *Cereal Chem.* 12: 377-389.
- Fairbanks, B. W., and Mitchell, H. H.
1935 The nutritive value of skim-milk powders with special reference to the sensitivity of milk proteins to heat. *J. Agr. Research* 51: 1107-1121.
- French, R. B., and Mattill, H. A.
1935 The biological value of the proteins of white, wheat, and rye breads. *Cereal Chem.* 12: 365-371.
- Gault, L. V.
1923 Yeast bread compared with baking powder bread in nutritive value. *J. Home Econ.* 15: 689-696.
- Hartwell, G. A.
1924 An experimental study of brown and white bread in the diet of the rat. *Biochem. J.* 18: 1323-1362.
- McCollum, E. V., Simmonds, N., and Parsons, H. T.
1921 Supplementary protein values in foods. V. Supplementary relations of the proteins of milk for those of cereals and of milk for those of legume seeds. *J. Biol. Chem.* 47: 235-247.

- Mitchell, H. H., and Smuts, D. B.
1932 The amino acid deficiencies of beef, wheat, corn, oats and soybeans for growth in the white rat. *J. Biol. Chem.* 95: 263-281.
- Morgan, A. F., and Frederick, H.
1935 Vitamin B (B_1) in bread as affected by baking. *Cereal Chem.* 12: 390-401.
- Rose, M. S., MacLeod, G., and Bisbey, B.
1923-4 Maintenance values for the proteins of milk, bread-and-milk, meat and soybean curd in human nutrition. *Proc. Soc. Exptl. Biol. Med.* 21: 143-144.
- Sherman, H. C., and Hawley, E.
1922 Calcium and phosphorus metabolism in childhood. *J. Biol. Chem.* 53: 375.
- Taylor, A. E.
1929 The place of wheat in the diet. *Wheat Studies*, Food Research Inst. Stanford Univ. 5: No. 4.
- Veselkin, N. V., Yaroslavtzeva, O. P., Seliber, G. L., and Bovshilk, G. A.
1927 Study of wheat bread and of brewer's and baker's yeast from the point of view of their vitamin B content, and an attempt at the preparation of a bread containing sufficient vitamins. *Bull. Inst. Lesshaft* 12: 87-96.

THE EFFECT ON FLOUR STRENGTH OF INCREASING THE PROTEIN CONTENT BY ADDITION OF DRIED GLUTEN¹

T. R. AITKEN and W. F. GEDDES

Board of Grain Commissioners, Grain Research Laboratory, Winnipeg, Canada

(Read at the Annual Meeting, May 1937)

Introduction

In a preceding paper, the authors (1934) showed that with a series of high grade Canadian hard red spring wheats, a linear relationship existed between protein content and loaf volume, from which they concluded that when wheats of the same class are under consideration protein content is a reliable index of strength.

Blish and Sandstedt (1935) point out that there is "a general unwillingness to accept protein content as a trustworthy index to strength when wheats of different classes or varieties are under consideration, because variations in gluten quantity are alleged to be overshadowed by inherent differences in 'quality,'" and while recognizing that glutes from different sources vary in their characteristics, they express the view that "protein content and inherent flour strength are one and the same thing."

It is well known that world wheats such as English, Australian, Argentine and Canadian differ widely both in protein content and gluten characteristics and, in view of this, it has not heretofore been possible to determine whether the very wide differences in their inherent strength are a reflection of variations in protein content, gluten character or both. Were it possible accurately to increase the protein content of "weak" to that of "strong" wheat flours, by the addition of the corresponding glutens, the influence of protein "character," or as it is generally termed "gluten quality," on flour strength could be evaluated. The use of wet gluten for such a purpose offers experimental difficulties from the standpoint of accurately determining the amount necessary to add and the possibility of changes in physical characteristics during the time required for moisture and protein determinations. To overcome these difficulties, a method has been developed whereby dried gluten of flour-like fineness may be prepared which will reform wet gluten closely resembling its original physical properties. With such preparations from different flours, the in-

¹ Published as paper No. 127 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.

vestigation here reported has been conducted with a view to determining the relative importance of variations in protein content and protein "character" in relation to the baking strength of typical world wheats.

Experimental

PREPARATION AND PROPERTIES OF DRIED GLUTEN

As high temperatures denature gluten, rendering it incapable of reforming wet gluten and valueless for increasing baking strength, it is necessary to conduct the drying at comparatively low temperatures and with sufficient rapidity to preclude putrefaction. In order to accomplish this and to permit drying wet glutens prepared from different flours under identical conditions, the experimental drying cabinet for alimentary pastes described by Binnington and Geddes (1934) was utilized. The glutens were washed from flour-water doughs by kneading under frequent changes of tepid water until most of the starch was removed; no attempt was made to obtain a relatively starch-free product, as the presence of small quantities of starch appeared to facilitate drying. The wet gluten was divided into small pellets weighing about four grams, placed on waxed paper trays and dried in the macaroni cabinet at 32° C. in a circulating air flow of approximately 400 cubic feet per minute, from which the moisture was condensed on cooling coils. The gluten was dried to a horny-like mass in 24 hours and was reduced to flour-like particles in the Allis-Chalmers experimental mill. The final product was bolted through a 9- $\times$ silk and contained approximately 8% moisture and 60 to 70% protein, the yield varying from 10 to 20%, depending upon the type of flour.

In selecting the drying conditions adopted, the object was to employ as low a drying temperature as possible without putrefaction, and no attempt was made to ascertain whether higher temperatures could be safely used. Alsberg and Griffing (1927) showed that heating wet gluten at temperatures from 30 to 50° C. resulted in no impairment of swelling power; in fact, the evidence indicated a slight increase, and heat coagulation at the temperature of 32° C. employed in this study would not be expected to occur. The similarity in appearance and feel of corresponding wet and wetted-dried glutens was very striking but the latter were, if anything, slightly more elastic, more extensible and firmer.

In this preliminary study, extensive tests were not undertaken to secure a quantitative measure of differences in physical properties between the wet and wetted-dried glutens from corresponding flours; however, the extent of dispersion of the dried glutens in 0.05N acetic acid was found to be in the order of 6% lower than the corresponding

freshly prepared glutens, this variation being practically constant for the different samples tested. It would therefore appear that the drying had slightly altered the gluten, but as the extent of the changes appeared to be uniform, the material was considered satisfactory for the purpose of this study.

EXPERIMENTAL MATERIAL AND METHODS

Twelve world wheats of widely varying baking strength, representing the strong, filler and weak classes, were experimentally milled into long patent flours, portions of which were used for preparing dried glutens as described. The flours and dried glutens were tested for moisture and protein content, and the necessary quantity of corresponding dried gluten added to each flour to raise the protein level to that of the highest in the series (Canadian Average No. 1 Northern), the flour-gluten blends being thoroughly mixed in a MacLellan mixer. In this manner, two series of samples were provided, the one varying in both protein content and gluten character, and the other only in the latter. In addition, the protein contents of two of the weakest flours in the series, namely, German and Italian, were raised to that of Canadian by the addition of No. 1 Northern gluten, thus permitting a direct comparison between the strength of so-called "weak" flours plus "weak" gluten and "weak" flours plus "strong" gluten of the same protein level. The flours investigated, together with protein data, are listed in Table I.

For the purpose of determining the comparative baking strengths of the original and gluten-treated flours, the samples were baked by the malt-phosphate-bromate method in which 0.3 g. diastatic malt (approximately 250° Lintner), 0.1 g. $\text{NH}_4\text{H}_2\text{PO}_4$, and 0.001 g. KBrO_3 are superimposed upon the ingredients specified in the A. A. C. C. Basic Baking Test outlined by Geddes (1934).

While it is admitted that flours possessing such widely varying strength characteristics as those included in this investigation undoubtedly possess different optimum fermentation and proving times, little definite information is available to indicate what these times should be. Experience (Aitken and Geddes, 1934; Kent-Jones and Geddes, 1936) has shown that of numerous formulas the malt-phosphate-bromate procedure widely differentiates between flours of varying and of similar strength characteristics and, accordingly, this baking procedure was used in the present study.

To determine the effect of additions of dried gluten on the type of curve produced by the Brabender Farinograph, farinograms were made on flour-water doughs at a consistency of 600 Brabender units employing 50 g. of flour (13.5% moisture basis).

TABLE I
FLOURS STUDIED AND PROTEIN DATA ¹

Reference number	Sample	Flour protein	Protein in dried gluten	Dried gluten added	Final flour protein
		%	%	%	%
<i>Original and corresponding gluten-treated flours</i>					
1	Canadian Average No. 1 Northern	13.7	63.4	nil	13.7
2	Canadian Average No. 2 C.W. Garnet	11.2	66.1	4.25	13.8
3	Canadian Average No. 5 (frosted)	12.9	68.4	1.34	13.7
4	Russian	12.5	67.6	2.03	13.9
5	German	8.4	70.6	7.92	13.6
6	Hungarian	11.7	66.8	3.37	13.8
7	English, ordinary	8.0	68.9	8.70	13.7
8	English, Yeoman	8.0	69.4	8.66	13.7
9	Argentine	10.5	65.6	5.43	13.9
10	Argentine	12.1	66.4	2.76	13.9
11	Australian	9.2	69.7	6.99	13.6
12	Italian	10.5	65.9	5.38	13.7
<i>Original and Canadian gluten-treated flours</i>					
13	German (No. 5)	8.4	63.4	10.40	13.9
14	Italian (No. 12)	10.5	63.4	6.90	13.8

¹ All values are expressed on a 13.5% moisture basis.

Baking Results

From the mean baking data recorded in Table II, it will be noted that the addition of dried gluten markedly increased absorption, improved dough handling characteristics, increased loaf volume and enhanced loaf appearance and crumb texture but imparted a greyish yellow colour to the crumb.

Regarding absorption, that of the original flours varied from 51.6 to 61.6% as compared with 55.6 to 63.6% for the gluten-treated flours, individual increases being as great as 5% in the instance of the lowest protein flours. While the range in absorption is only narrowed by 2% by equalizing the protein content, the majority of the gluten-treated flours have absorptions ranging from 58.6 to 60.6%, the outstanding exceptions being ordinary English, German and Italian.

In dough-handling characteristics, the improvement due to the added gluten was very striking, particularly with the English, Italian and German, which normally yield short, sticky, tender and inelastic doughs; the corresponding treated flour doughs exhibited medium strength dough characteristics, similar to those of a good Argentine.

TABLE II
EAN FLOUR PROTEIN AND COMPARATIVE BAKING DATA

Reference number	Sample	Flour protein I	Ab-sorp-tion-vol-ume	Crumb score		Crust colour	Form	Dough characteristics at time of panning
				Texture	Colour			
1	Canadian Average No. 1 Northern	13.7	60.6	6	7 y.	S.	4.5	Excellent elasticity and spring; very gassy; exceedingly "strong."
2	Canadian Average No. 2 C. W. Garnet	11.2	56.6	618	4.5 EG.	S.	2 sl. 3.	Only fair elasticity and spring; somewhat short. Medium strength.
2 G. ²	Canadian Average No. 2. C. W. Garnet plus gluten	13.8	59.6	738	3.5 EG.	S.	4	Good elasticity and spring; not short. Markedly superior to No. 2. Very good strength.
3	Canadian Average No. 5	12.9	735	735	6.5 y.	S.	3 s.	Good spring and elasticity; somewhat tender. Good to medium strength.
3 G.	Canadian Average No. 5 plus gluten	13.7	82.6	765	5.5 C. and o.	S.	3 s.	Very good strength; closely resembles Average No. 1 Northern in all respects.
4	Russian	12.5	728	728	5.5 C. and o.	S.	3 s.	Good elasticity and spring; somewhat tough. Good medium strength.
4 G.	Russian plus gluten	13.9	59.6	775	7 y.	S.	4	Equal to Average No. 1 Northern in all respects.
5	German	8.4	51.6	525	5 Cl.	VP.	1.5 s.	Very short and clay-like.
5 G.	German plus gluten	13.6	55.6	640	7 g.	S.	4	Shortness absent; marked improvement in spring and elasticity. Medium strength.
5 CG. ³	German plus Average No. 1 Northern gluten	13.9	57.6	715	7	S.	4.5	Very similar to Average No. 1 Northern in all respects.
6	Hungarian	11.7	58.6	695	7.5 y.	S.	3 s.	Very fair elasticity and spring; somewhat tender. Medium strength.
6 G.	Hungarian plus gluten	13.8	60.6	795	6.5	S.	4	Very similar to Average No. 1 Northern in all respects.
7	English, ordinary	8.0	53.6	455	3 C.	P.	1 f.	Lifeless; very short and clay-like. Very poor strength.
7 G.	English plus gluten	13.7	57.6	595	5 Cl.	S.	3 s.	Fair elasticity and spring; very great improvement in strength when compared with No. 7.
8	English Yeoman	8.0	55.6	522	4 C.	P.	1 f.	Clay-like, rather short and tender. Poor strength.
8 G.	English Yeoman plus gluten	13.7	60.6	725	5.5 C. and o.	S.	3 s.	Shortness absent; very fair elasticity and spring; resembles good Argentine.
9	Argentine	10.5	55.6	692	7	S.	4	Fair spring and elasticity; somewhat tender. Medium strength.
9 G.	Argentine plus gluten	13.9	59.6	815	6.5 EG.	S.	4	Very similar to Average No. 1 Northern in all respects.
10	Argentine	12.1	56.6	675	6.5	S.	4.5	Similar to No. 9.
10 G.	Argentine plus gluten	13.9	58.6	725	6.5 EG.	S.	4.5	Very similar to Average No. 1 Northern in most respects; somewhat less springy.
11	Australian	9.2	53.6	535	5 Cl.	P.	2	Short, clay-like and tender. Fair strength only.
11 G.	Australian plus gluten	13.6	58.6	732	7	S.	4.5	Closely resembles Average No. 1 Northern but somewhat tender.
12	Italian	10.5	61.6	550	4 C.	S.	2	Slack tender, somewhat sticky.
12 G.	Italian plus gluten	13.7	63.6	695	4.5 C.	D.	2	Fairly springy; practically no stickiness, rather like medium quality Argentine.
12 CG.	Italian plus Average No. 1 Northern gluten	13.8	63.6	708	3 y.	D.	4	Very similar to good Argentine.

¹ 13.5% moisture basis.

² G. = Gluten.

³ C. = Canadian.

Table abbreviations

{Crumb texture: (Perfect score 10). Cl. = Coarse; C. = Close; o. = open.

{Crust colour: (Perfect score 10). g. = grey; y. = yellow; V. = Very.

{Crust colour: (Perfect score 10). P. = Pale; D. = Dark; V. = Very.

{Form: (Perfect score 5). s. = shell-top; f. = flat; sl. = slightly.

English Yeoman, Australian and Argentine were "transformed" to medium strength "Manitoba" doughs, such as Atlantic No. 3 Northern.

In loaf volume, the addition of dried gluten, as shown in Figures 1 and 2, imparted marked improvement, particularly to the flours of low protein content. The loaf volumes for the untreated flours ranged from 455 c.c. to 925 c.c., a spread of 470 c.c., as compared with

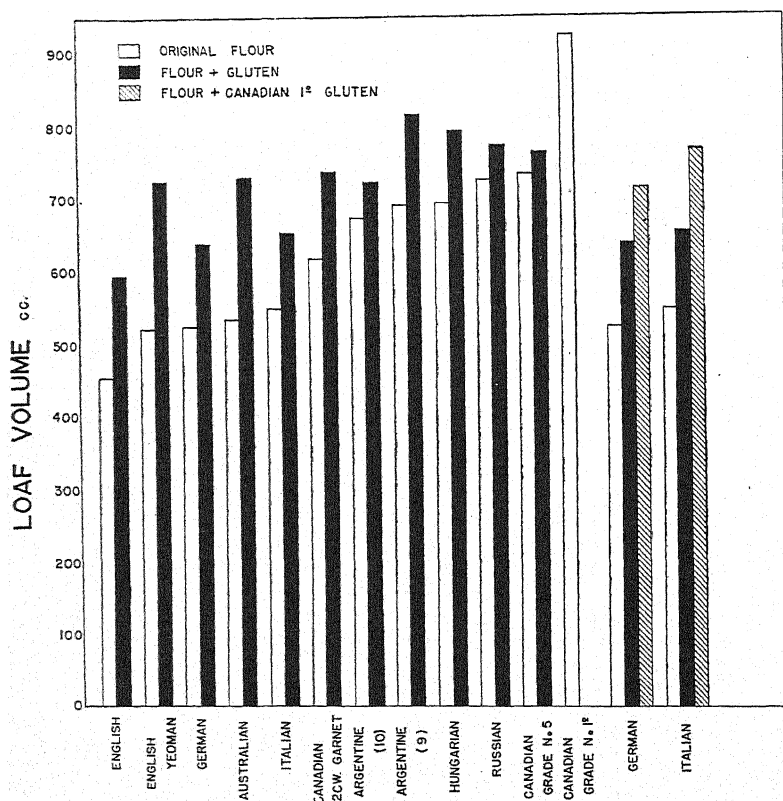


Figure 1. Histogram showing the comparative loaf volumes for the original and gluten-treated flours.

595 to 925 c.c., or a spread of 330 c.c., for the gluten-treated flours; that is, by equalizing the protein content the spread has been reduced by 140 c.c. or approximately 30%. However, this hardly gives a fair representation of the general improvement in strength, since, omitting the three weakest samples, namely, ordinary English, German and Italian and the very strong Canadian No. 1 Northern, the loaf volumes for the remaining eight gluten-treated flours all fell within a range of 90 c.c. as compared with 213 c.c. for the original flours. Within this

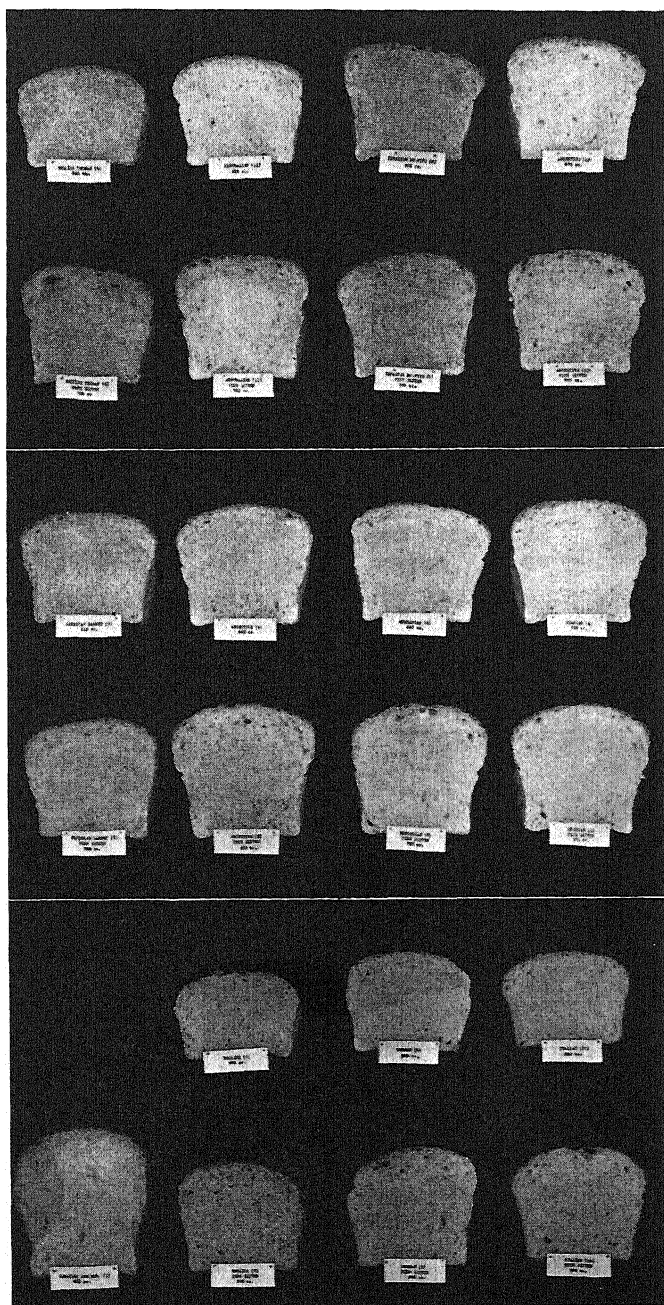


Figure 2. Photographs showing the cut surfaces of loaves baked from the various flours with and without additions of the corresponding dried glutens.

group, five of the treated flours, namely, English Yeoman, Australian, Garnet, Argentine and Russian gave loaf volumes of between 725 c.c. and 775 c.c., as compared with 522 to 728 c.c. or a spread of 200 c.c. for the original flours. From this it would appear that, with the exception of the types of wheat which are considered to be representative of extremes as regards baking potentiality, namely, weak European and strong Canadian, protein content is the most important factor affecting baking strength; in other words, wheats such as average quality English Yeoman, Australian, Garnet, Argentine, Hungarian, Russian and Canadian No. 5 frosted would appear to possess protein having similar strength-imparting characteristics.

However, since any changes induced in the glutes as a result of drying appeared to be uniform for all samples, there is fairly definite evidence that baking strength is in part influenced by so-called "gluten quality." While none of the treated flours yielded loaves equal in volume to the strong Canadian, the fact that the latter sample contained no added gluten renders this less conclusive than the relative results obtained by adding "strong" versus "weak" gluten to the German and Italian flours. The addition of their own glutes increased the loaf volume of the German and Italian flours by 115 c.c. and 105 c.c. respectively, whereas the Canadian gluten caused increases of 190 c.c. and 218 c.c., as illustrated in Figures 1 and 3.

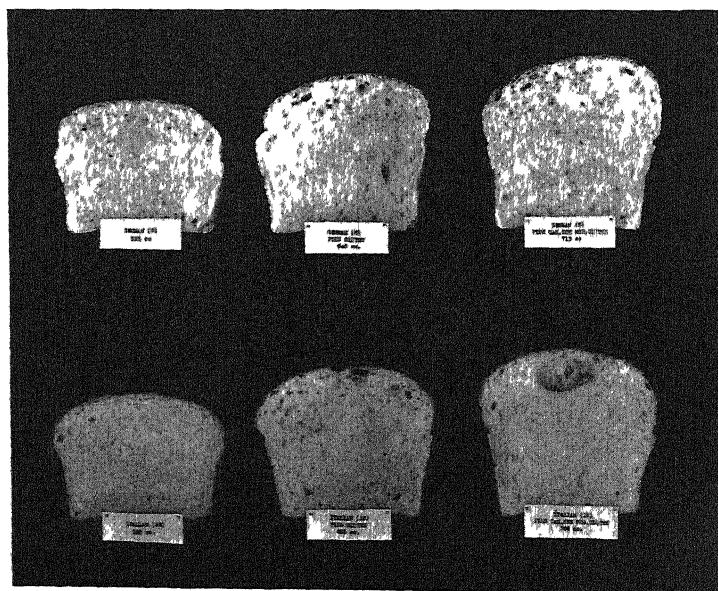


Figure 3. Photograph showing the cut surfaces of loaves baked from German and Italian flours together with those for these flours with additions of their own versus Canadian dried gluten

With reference to other loaf characteristics, the external appearance was markedly improved, the loaves being more "bold" and possessing superior break and shred, by the gluten additions. In internal characteristics the crumb grain and texture were not impaired and in many cases were improved, but the crumb colour was inferior, the gluten-treated flours yielding loaves possessing a decided yellow or greyish-yellow colour. This undesirable feature could undoubtedly be minimized by preparing the glutes from bleached flours, as the carotenoid pigments are to a large extent adsorbed by the gluten during the washing process.

TABLE III

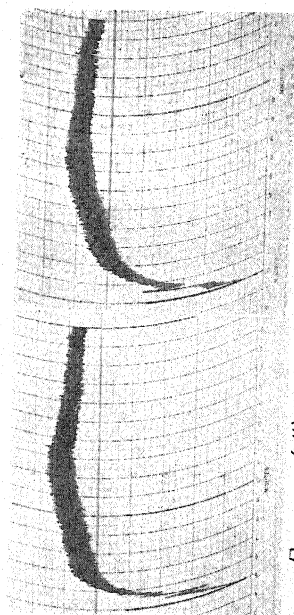
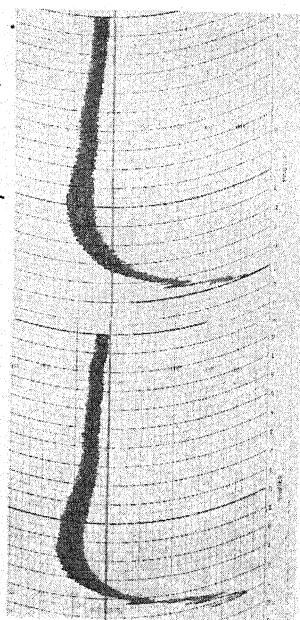
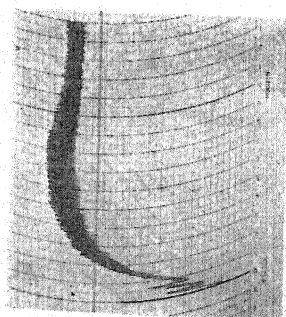
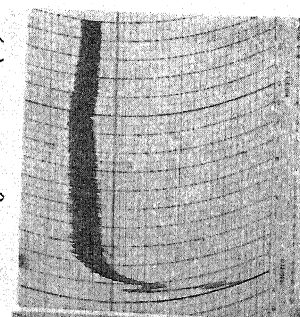
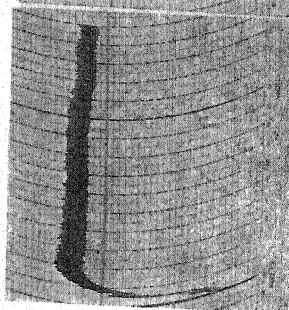
COMPARATIVE DOUGH DEVELOPMENT TIME AND WATER ABSORPTION
OF FLOURS WITH AND WITHOUT ADDITIONS OF DRIED GLUTEN

Refer- ence number	Sample	Ab. sorp- tion ²	Dough develop- ment time
		%	Minutes
1	Canadian Average No. 1 Northern	58.4	7.5
2	Canadian Average No. 2 C. W. Garnet	54.6	2.0
2 G. ¹	Canadian Average No. 2 C. W. Garnet plus gluten	56.4	8.0
3	Canadian Average No. 5 (frosted)	59.0	3.5
3 G.	Canadian Average No. 5 (frosted) plus gluten	60.4	5.5
4	Russian	55.6	7.5
4 G.	Russian plus gluten	57.6	7.5
5	German	49.8	1.0
5 G.	German plus gluten	53.8	2.5
5 CG. ²	German plus Average No. 1 Northern gluten	55.8	5.5
6	Hungarian	56.6	3.0
6 G.	Hungarian plus gluten	59.0	3.0
7	English, ordinary	51.2	0.5
7 G.	English plus gluten	55.2	2.5
8	English Yeoman	52.4	1.5
8 G.	English Yeoman plus gluten	57.4	3.5
9	Argentine	54.0	3.5
9 G.	Argentine plus gluten	58.0	5.0
10	Argentine	54.8	2.5
10 G.	Argentine plus gluten	56.4	2.5
11	Australian	52.0	1.5
11 G.	Australian plus gluten	56.4	3.5
12	Italian	63.2	2.0
12 G.	Italian plus gluten	65.4	3.0
12 CG.	Italian plus Average No. 1 Northern gluten	63.2	4.0

¹ G. = corresponding gluten.

² CG. = Canadian No. 1 Northern gluten.

³ 13.5% moisture basis.

*Russian plus Gluten.**Russian (*4)**Argentine plus Gluten.**Argentine (*9)**Canadian Average One Northern (*4)**Canadian Ave. Garnet plus Gluten.**Canadian Ave. Garnet (*2)*

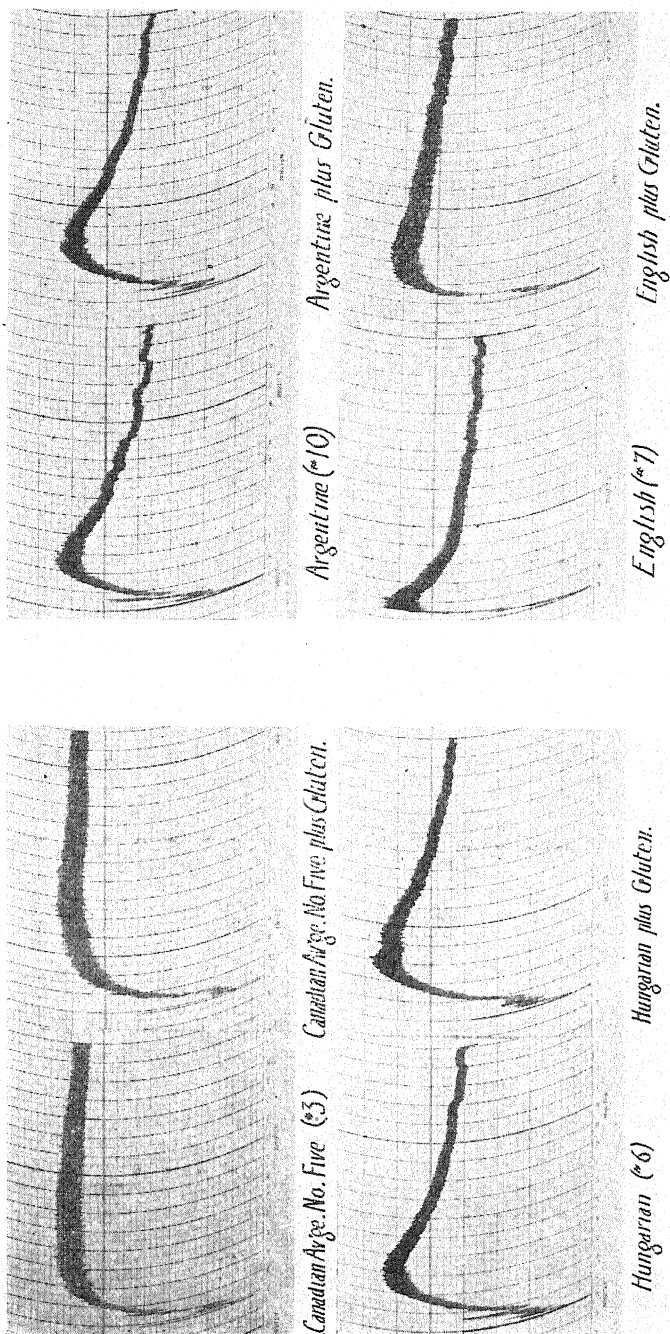


Figure 4. Farinograms for the original and gluten treated flours.

Figure 4 continued on page 192.

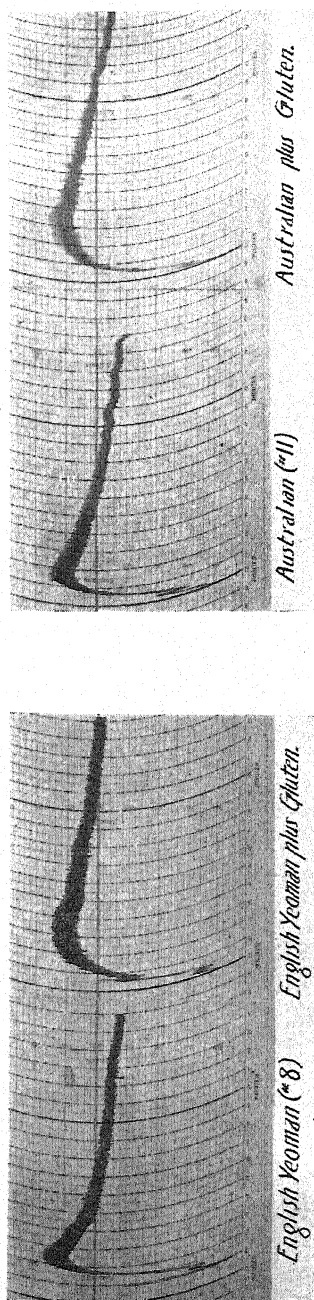


Figure 4 (continued). Farinograms for the original and gluten-treated flours.

Farinograph Results

The farinograms obtained are reproduced in Figures 4 and 5, and the absorptions required to bring the doughs to a Brabender consistency of 600 units, together with the "dough development time" in minutes as read from the curves, are given in Table III.

The water absorption and "dough development time" were, in general, increased by the gluten additions.

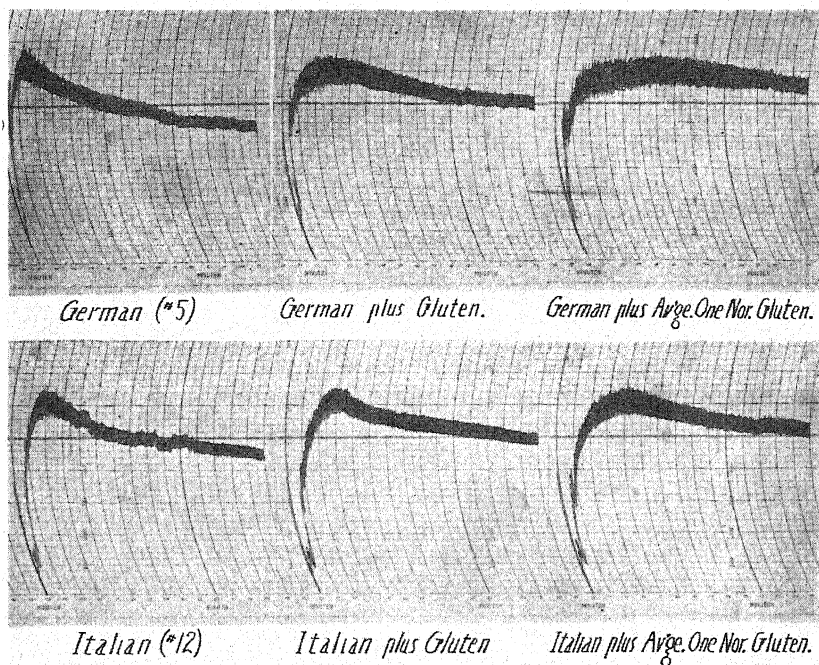


Figure 5. Farinograms for German and Italian flours, and those for these flours with additions of their own versus "Canadian" dried gluten.

Considering the general type and shape of the corresponding curves, it is evident that in the majority of cases improvement resulted from raising the protein level by the added gluten. This is particularly noticeable in the case of the English, English Yeoman, Australian and Italian flours which, as evidenced by curve appearance, were transformed from weak flours to those of the Argentine type. The improvement in the German and Italian flours due to the addition of Canadian gluten is most striking, the final curves resembling respectively those from a medium strength Canadian and a good Argentine. Neither the Russian, Hungarian nor Argentine curves were noticeably altered by the gluten additions.

The farinograms for the gluten-treated flours, in general, showed less improvement in flour strength than that evidenced by the loaf volume data and in several instances flours of the same protein and loaf volume levels gave quite different types of curves. Considering the curves for the gluten-treated flours as a whole, it is quite obvious that the dough characteristics as measured by the farinograph are not merely a reflection of protein content.

Effect of Storage on the Keeping Quality of Dried Gluten

In order to determine whether deterioration takes place in the strength-imparting properties of dried gluten after storage, additional baking tests were made with several of the glutes which had been stored in capped bottles for approximately ten months.

For this purpose, four of the original wheats, *i.e.*, Italian, German, Garnet and Argentine, were experimentally milled to the same extraction as formerly, care being taken in the milling to provide a second lot of flours which would be essentially the same as those formerly milled; protein tests showed a variation of only 0.1% between corresponding samples. Portions of the original dried glutes were added to the freshly milled corresponding flours to raise the protein content to the level originally selected, *i.e.*, 13.7%, thus permitting comparisons to be made between their baking strength and that of the original gluten-treated flours. The loaf volume results obtained by the malt-phosphate-bromate procedure and those by the same baking method originally found are shown in Table IV.

As indicated by the corresponding loaf volume increases, it is evident that dried gluten did not deteriorate after ten months' storage.

Discussion

This preliminary study reveals that dried gluten can readily be prepared which will reform wet gluten closely resembling freshly washed gluten in general handling properties and, when added to a weak flour, will impart strong flour characteristics, as indicated by increased water absorption, improved dough handling quality, loaf volume, loaf appearance and type of farinogram. There are definite indications that certain types of flours such as those milled from English Yeoman, Australian, Argentine, Russian, Hungarian, Canadian frosted and Garnet wheats differ in baking strength chiefly on account of variations in protein content, and that with such types "protein character" is of lesser importance.

On the other hand, with flours milled from the weakest types of wheats such as English, German and Italian, and from very strong types, such as high grade Canadian, definite differences in "protein

TABLE IV
LOAF VOLUME DATA OF FLOURS PLUS GLUTENS BEFORE AND AFTER
APPROXIMATELY TEN MONTHS' STORAGE OF THE GLUTENS

Reference number	Sample	Original loaf volume		Loaf volume 10 months' later	
			Increase due to gluten addition		Increase due to gluten addition
2	Canadian Average No. 2 C. W. Garnet	C.c.	%	C.c.	%
2 G.	Canadian Average No. 2 C. W. Garnet plus gluten	618	—	662	—
10	Argentine	738	19.5	772	16.6
10 G.	Argentine plus gluten	675	—	705	—
12	Italian	725	7.4	758	7.5
12 G.	Italian plus gluten	550	—	542	—
5	German	655	19.1	632	16.6
5 G.	German plus gluten	525	—	572	—
		640	21.9	712	24.5

character" were evidenced. In other words, the weakest wheats are "weak" not only because of low protein content but also because of the inferior character of the protein or gluten. Similarly, the superior strength of Canadian No. 1 Northern is attributable to both high protein content and superior strength-imparting properties of its gluten. While increasing the protein content of weak flours by the addition of their own dried gluten resulted in marked general improvement, the addition of "strong gluten" produced more pronounced beneficial effects. Weak flours like English, German and Italian, which normally yield doughs of poor handling quality, take on "Manitoba" characteristics as shown by improvement in elasticity, spring and stability.

The question naturally arises as to whether any difference exists in the strength-imparting properties of wet and dried gluten from the same source and if subsequent tests indicate that the glutens are essentially the same in this regard, or that the dried glutens from various sources are modified to the same extent, a technique is provided for measuring differences in the gluten character of different grades of flour, varieties, grades and classes of wheat.

Summary

By employing a low drying temperature of 32° C. and a rapid air flow, dry gluten has been prepared which, after reducing to flour-like

fineness, yields wet gluten closely resembling freshly prepared wet gluten from corresponding flours in the major physical properties as indicated by hand manipulation. Limited tests indicated that the degree of dispersion in 0.05N acetic acid has been somewhat lowered as a result of drying.

Dried glutens prepared from experimentally milled flours representing weak, intermediate and strong world wheats, when added to the corresponding flours to equalize their protein contents, resulted in decided improvement in flour strength as indicated by absorption, dough-handling quality, loaf volume and loaf appearance. The crumb texture of the gluten-treated loaves was either unimpaired or improved but the crumb colour was deleteriously affected, being decidedly more yellow or greyish-yellow.

There are indications that wheats like English Yeoman, Australian, Hungarian, Argentine, Russian, Canadian frosted and Garnet possess glutens of similar character, and that their varying strength characteristics are due primarily to differences in protein content; with wheats of this type, protein content is a satisfactory index of strength. Wheats such as ordinary English, German and Italian are essentially "weak" because of deficient protein content coupled with gluten of unsatisfactory, weak character; on the other hand, high grade, high protein Canadian wheat owes its superior baking potentiality to its high protein level combined with gluten of good "quality."

Literature Cited

- Aitken, T. R., and Geddes, W. F.
1934 The behaviour of strong flours of widely varying protein content when subjected to normal and severe baking procedures. *Cereal Chem.* 11: 487-504.
- Alsberg, C. L., and Griffing, E. P.
1927 The heat coagulation of gluten. *Cereal Chem.* 4: 411-423.
- Binnington, D. S., and Geddes, W. F.
1934 Experimental drying equipment for alimentary pastes. *Can. J. Research* 10: 221-233.
- Blish, M. J., and Sandstedt, R. M.
1935 Definition and measurement of "flour strength" as an inherent property of wheat. *Cereal Chem.* 12: 653-664.
- Geddes, W. F.
1934 Official A. A. C. C. basic baking test. *Cereal Chem.* 11: 363-367.
- Kent-Jones, D. W., and Geddes, W. F.
1936 A co-operative study of the utility of different methods for evaluating flour strength. *Cereal Chem.* 13: 239-280.

X-RAY PHOTOGRAPHS AS A MEANS OF DETERMINING WHEN A DOUGH IS ADEQUATELY MIXED

J. A. SHELLENBERGER

The Mennel Milling Company, Toledo, Ohio

(Received for publication July 29, 1937)

The standard experimental baking test as outlined in "Cereal Laboratory Methods" and "Methods of Analyses of the Association of Official Agricultural Chemists" specifies the use of the Hobart-Swanson mixer and a one-minute mixing period. The choice of a one-minute mix for the standard basic procedure is a necessary precaution because prolonging the mixing period beyond one minute will, in many cases, over-develop the gluten and thus adversely affect the baking properties of the dough. This is particularly true of doughs made from weak flours.

However, there has been some doubt concerning the mixing thoroughness when only a one-minute mixing period is used. The following investigation was undertaken to determine if a one-minute mix is sufficient to obtain a uniform distribution of the dough ingredients.

Method

The procedure adopted was as follows: One hundred grams of flour on a 15% moisture basis were placed in the bowl of the Hobart-Swanson mixer and a suspension of barium sulphate added plus sufficient water to produce a dough of the proper consistency. The quantity of barium sulphate added was $6\frac{1}{2}\%$ because this figure corresponds with the total amount of ingredients added in the form of yeast, sugar and salt in the standard baking test. Doughs were made up in this manner from cracker, baker's patent, and strong, clear-grade flours, and mixing periods varying from one to four minutes were investigated.

The procedure adopted for determining mixing thoroughness was to X-ray the doughs containing the barium sulphate. This was accomplished by rounding up the dough, as it comes from the mixer, in the same manner as though it were to be placed in the graniteware fermentation bowl. The rounded dough was placed on the sup-

porting plate of the X-ray machine and pressed to a uniform thickness by the use of a greased glass plate. The pictures are made by passing the X-rays directly down through the entire dough.

Figures 1, 2 and 3 are typical X-ray pictures of three doughs made from the same flour, the mixing period being the only variable. If after mixing the doughs were entirely homogeneous, the intensity of

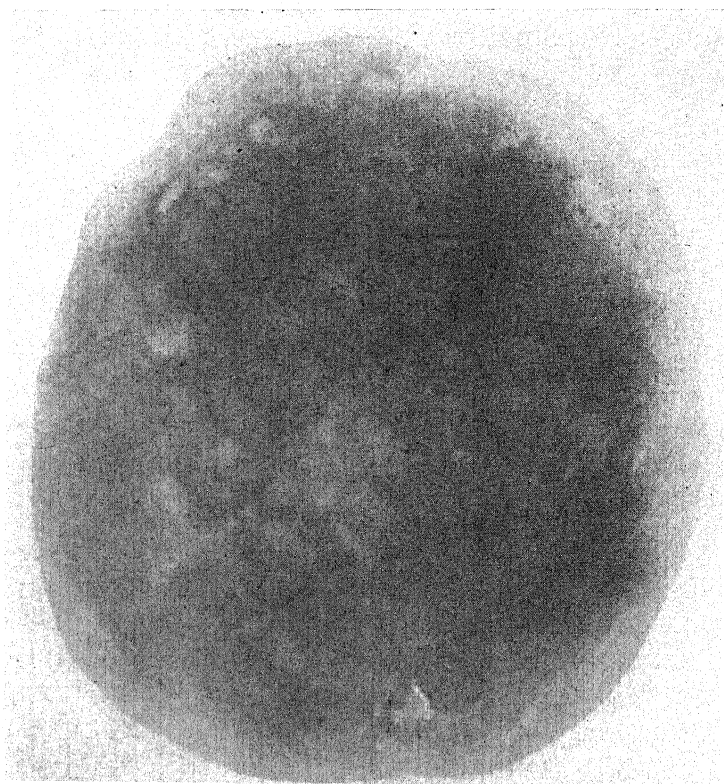


Figure 1. X-ray picture of a dough containing 6 $\frac{1}{4}$ % barium sulphate after a one-minute mix.

the shadow effect produced by the presence of the barium sulphate would be of equal intensity throughout the central portion since the X-rays are actually penetrating the same thickness of material. The appearance of the outer portion of the dough should be disregarded. In the present case a small quantity of larger pieces of barium sulphate was added in order that the mixing could be more easily followed.

When only powdered barium sulphate is used the shadow effect showing the lack of uniform distribution of the barium sulphate is sometimes difficult to see and requires a trained technician to properly interpret the picture.

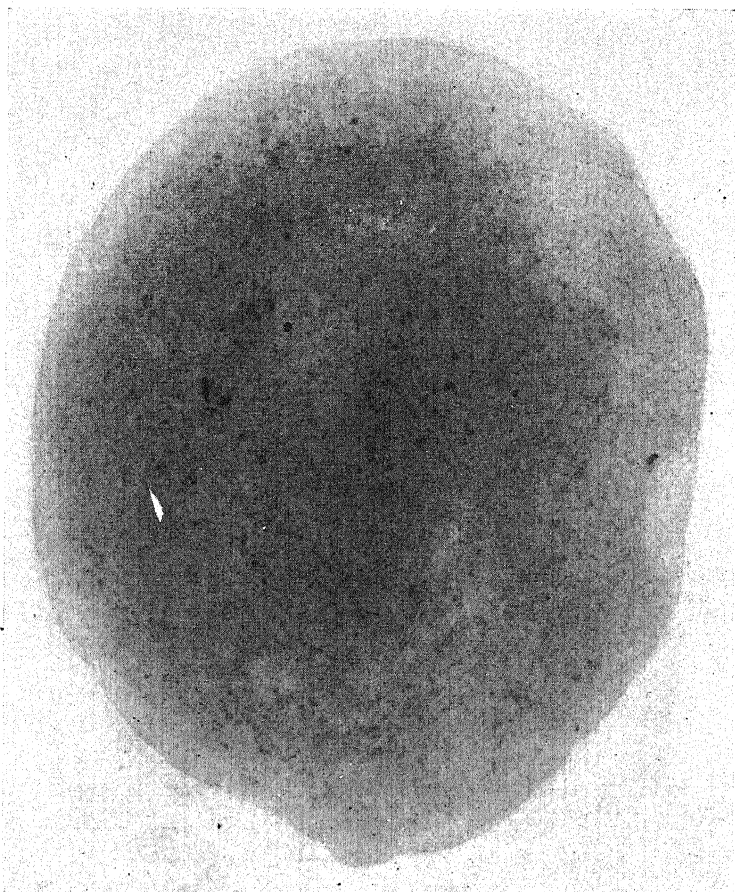


Figure 2. X-ray picture of the same dough in Figure 1 after being mixed two minutes.

These pictures indicate clearly that a one-minute mixing period is not adequate for thorough incorporation of the ingredients. Notice the grouping of the barium sulphate particles and the light shaded areas (Figure 1). Figures 2 and 3 show that the additional mixing has produced a uniform distribution of the barium sulphate.

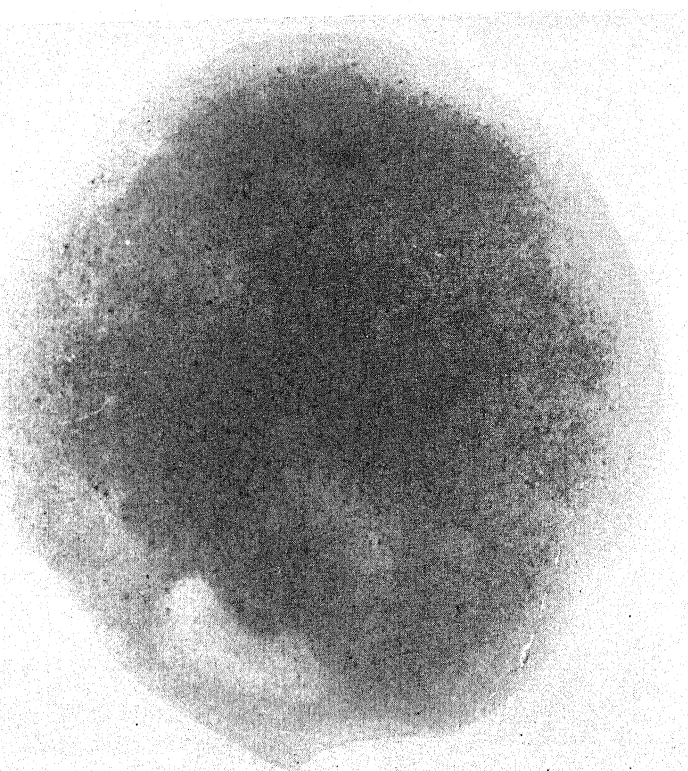


Figure 3. X-ray picture of the same dough in Figure 1 after being mixed three minutes.

Conclusions

If the properties of the flour will permit, it is advantageous to mix a dough in the Hobart-Swanson mixer for longer than one minute to completely incorporate the ingredients.

A two-minute mixing period was found to be sufficient for the thorough mixing of even very tough doughs.

THE ACTION OF ASCORBIC ACID AS A BREAD IMPROVER

JAMES MELVILLE and HAROLD T. SHATTOCK¹

The Wheat Research Institute, Christchurch, New Zealand

(Received for publication September 1, 1937)

In a paper dealing with a new conception of the role played by bromate and allied substances in panary fermentation, Jørgenson (1935) announced the discovery that ascorbic acid could act as a bread improver. In countries such as New Zealand where the addition of bromate to flour is illegal this discovery is of considerable potential importance since, although such improvers have to be used with caution on soft flours, there are occasions when the flour from certain areas or in certain seasons would benefit by their application.

During an investigation into the utility of lemon juice which is one of the cheapest and richest natural sources of ascorbic acid, it was discovered that the improving action did not decrease with age of the sample, despite the ready oxidizability of ascorbic acid by atmospheric oxygen. In one case a sample of lemon juice which was about six months old and which had practically no reducing power as measured by an iodine titration, was just as potent as a fresh sample. We were interested also in the apparent contradiction that whereas bromate, iodate, persulphate, etc., are strong oxidizing agents, ascorbic acid, with apparently the same action in panary fermentation, is a moderately strong reducing agent.

Experimental

Starting from the observation that lemon juice containing no reduced ascorbic acid was capable of acting in the same way as bromate, the first step was an investigation of the action of oxidized ascorbic or dehydroascorbic acid in panary fermentation. Dehydroascorbic acid was prepared by a simple iodine oxidation of ascorbic acid in aqueous solution, the oxidation being carried out immediately before addition to the dough. Results of volume determinations are given in Table I for two flours milled experimentally from New Zealand wheats and showing a large bromate response. The nitrogen figures for the two flours No. 53 and No. 203 were 2.40% and 2.24% respectively, figures considerably higher than the average for New Zealand wheats.

¹ National Research Scholar.

Only volumes are recorded in Table I, but other loaf characteristics exactly paralleled the differences in volume. Although results are given for only two flours, experiments with other flours with a large bromate response led to very similar results.

It may be seen from this table that bromate and dehydroascorbic acid have approximately the same improving action, weight for weight basis, and that dehydroascorbic acid is much more efficacious than is reduced ascorbic acid. In view of the easy oxidizability of ascorbic acid it was felt that these data were most easily explained on the hypothesis that ascorbic acid acts as a bread improver only in its oxidized form and that there exists in flour a mechanism whereby this oxidation can be easily effected.

In 1931 Szent-Györgyi showed that there exists in cabbage leaves an enzyme which he named hexoxidase and which acted as a catalyst in bringing about the change of ascorbic to dehydroascorbic acid in the

TABLE I

125 g. of flour, 2.5 g. of yeast, 2.5 g. of sugar, 1.8 g. of salt. 3 minutes mixing on a Hobart mixer. 4 hours 5 minutes from mixer to oven. Volume in cubic centimeters.

Laboratory Number	203	53
Basic formula	540	510
+ 5 mg. bromate	740	700
+ 5 mg. ascorbic acid	650	630
+ 5 mg. dehydroascorbic	730	720
+ 20 mg. ascorbic	700	—

presence of oxygen. Since that time it has been shown by Srinivasan (1936) and by Johnson and Zilva (1937) that hexoxidase or ascorbic acid oxidase is very widely distributed in nature and its presence in flour seemed quite probable.

To demonstrate this, straight-run flour was stirred into a paste with twice its weight of water, left overnight in an ice chest, the suspension centrifuged, and 50 c.c. of the supernatant liquid added to 6 mg. of ascorbic acid dissolved in 25 c.c. of water. The course of oxidation was followed by titration with 2 : 6 dichlorophenol indo-phenol, and it was found that after seven hours 89% of the ascorbic had been oxidized.

Concentration of the enzyme, using the directions of Szent-Györgyi, was then attempted. Thirteen hundred cubic centimeters of a flour-water extract were treated with ammonium sulphate to 30% saturation and the precipitate centrifuged off. The precipitate contained negligible amounts of ascorbic acid oxidase. The liquor was saturated with ammonium sulphate and the precipitate dissolved in

100 c.c. of M/15 phosphate buffer at pH 5. Fifty cubic centimeters of this solution were added to 10 mg. of ascorbic acid in 50 c.c. of water and the oxidation was followed by titration with 2 : 6 dichlorophenol indophenol. The rate of oxidation is given in Table II.

TABLE II
RATE OF OXIDATION OF ASCORBIC ACID

Time in hours	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$
Titre	5.10	4.54	3.96	3.42	2.77	2.15	1.63	1.05

Hence about 80% of the ascorbic acid was oxidized in $3\frac{1}{2}$ hours, and the rate of oxidation is a linear function of time, a property of the enzyme clearly demonstrated by Hopkins and Morgan (1936). These same authors also show that ascorbic acid oxidase from cauliflower is inhibited by very small concentrations of cyanide, and it was found that the enzyme prepared from flour exhibits the same phenomenon. In the presence of M/1000 cyanide, with all other conditions the same as in the experiment already described, only 15.5% of the ascorbic had been oxidized at the end of four hours.

One further point remained to be proved, *viz.*, that the enzyme is capable of functioning as an oxidation catalyst during fermentation. For this purpose a concentrated enzyme solution was prepared from the florets of cauliflower, concentration and purification from any ascorbic acid which might be present being effected by fractionation with ammonium sulphate. A solution was used which had been kept for a week in the icebox and whose potency was such that 2 c.c. oxidized 5 mg. of ascorbic in 45 minutes.

TABLE III
RESULTS WITH FLOUR NO. 203

Flour number	Loaf volume in c.c.
203	540
203 + 5 mg. ascorbic	650
203 + 5 mg. iodine oxidised ascorbic	730
203 + 10 c.c. enzyme solution	540
203 + 5 mg. ascorbic + 10 c.c. enzyme	730
203 + 5 mg. enzyme oxidized ascorbic ¹	740

¹ In this experiment the ascorbic was oxidized by ascorbic acid oxidase before being added to the dough.

Reversibly and Irreversibly Oxidized Ascorbic Acid

The instability of dehydroascorbic acid in aqueous solution at reactions higher than pH 4 has been stressed by a number of investi-

gators, and the irreversible and spontaneous change of dehydroascorbic acid to diketogulonic acid has been studied in detail by Borsook *et al.* (1937). In an endeavour to elucidate further the mechanism of ascorbic acid improvement the effect of diketogulonic acid was tried. The directions of Borsook for the preparation of this material, *viz.*, incubation of dehydroascorbic at pH 7 and 37° C., were followed. Reduction with hydrogen sulphide showed that only 5% of dehydroascorbic acid originally present remained in the reaction mixture.

The addition of this material to the dough gave no perceptible change in volume as compared with the control, so that the improving action can be narrowed down to the components of the reversible redox system of ascorbic-dehydroascorbic.

Discussion

The demonstration that dehydroascorbic acid is, on certain flours, a more efficient improver than is ascorbic acid, and that there exists in flour a mechanism whereby the oxidation of ascorbic to dehydroascorbic can take place, lends support to the hypothesis that ascorbic acid *per se* is inactive and that the improving action of this material is due entirely to its oxidation product. It will be noted that the two flours reported in Table I require comparatively high quantities of bromate in order to produce the best loaves of which they are capable, and that in these cases the effect of dehydroascorbic acid can not be duplicated by four times the amount of ascorbic acid. The case with flours requiring less bromate than 4 mg. per percent is otherwise. The difference between ascorbic acid and its oxidation product becomes much less marked and in certain cases disappears entirely. From experiments carried out on a range of flours, the only conclusion at which we have been able to arrive is that dehydroascorbic acid is always as potent as ascorbic acid and in the majority of cases is considerably more potent. On the basis of the hypothesis outlined above, this is not surprising since there are a number of variables involved in the oxidation, *e.g.*, variations in ascorbic acid oxidase content of different flours, which could account for such discrepancies.

Nevertheless the evidence, although suggestive, is by no means conclusive. We have extended our investigations, in an endeavour to test Jørgenson's theory of the effect of improvers on the proteolytic enzymes of flour, to the effect of ascorbic and dehydroascorbic acids on papain. While recognizing that such experiments are open to objections, it is of interest that preliminary results indicate that both ascorbic and dehydroascorbic acids inhibit papain, the latter to a greater degree than the former.

Summary

Dehydroascorbic acid is a bread improver which is equivalent to bromate on a weight for weight basis. On flours requiring relatively large quantities of bromate dehydroascorbic acid is more potent than ascorbic acid.

It has been shown that flour contains the enzyme ascorbic acid oxidase which is capable of catalyzing the oxidation of ascorbic acid to dehydroascorbic acid.

Literature Cited

- Borsook, H., Davenport, H. W., Jeffreys, C. E. P., and Warner, R. C.
1937 The oxidation of ascorbic acid and its reduction *in vitro* and *in vivo*.
J. Biol. Chem. 117: 237-279.
- Hopkins, F. G., and Morgan, E. J.
1936 Some relations between ascorbic acid and glutathione. Biochem. J.
30: 1446-1462.
- Johnson, S. W., and Zilva, S. S.
1937 The oxidation of l-ascorbic acid by plant enzymes. Biochem. J. 31:
438-453.
- Jørgenson, H.
1935 The nature of bromate action. Das Mühlenlaboratorium 5: 114.
- Srinivasan, M.
1936 Ascorbic acid oxidase from drumstick. Biochem. J. 30: 2077-2084.
- Szent-Györgyi, A.
1931 On the function of hexuronic acid in the respiration of the cabbage leaf.
J. Biol. Chem. 90: 385-393.

OVEN SPRING OF DOUGH AS CORRELATED WITH CERTAIN PROPERTIES OF BREAD ¹

W. O. WHITCOMB

Montana Agricultural Experiment Station, Bozeman, Montana

(Read at the Annual Meeting, May 1937)

At the last annual meeting it was shown how the oven spring of dough was influenced by varying amounts of sugar, salt, and yeast. The present paper shows how oven spring is related to certain properties of bread when the sugar, salt, and yeast are held constant.

Materials

The wheats used in this study were varieties of hard red spring and hard red winter of the crop years 1933 and 1934. These included the more important recognized varieties together with a number of selections and crosses in both the spring and winter wheats. The varieties of the 1933 crop of spring wheats were Marquis, Ceres, Supreme, Red Bobs, Marquillo, Reliance, Komar, Hope, Reward, and Kota, while those of the 1934 spring wheat were Marquis, Supreme, Ceres, Thatcher, Hope, Reliance, and Reward. Because of the limited number of lots of winter wheat available, the crops of 1933 and 1934 were combined for the study. These varieties were Turkey, Nebraska No. 60, Karmont, Newturk, Yogo, Cheyenne, Minard, Montana 36, and Oro.

These wheats were grown in three locations in Montana under varying conditions of moisture as found on dry land and under irrigation. They were all well matured, were not subjected to freezing before harvest, nor were they damaged by sprouting in the field or by severe weathering. The weight per bushel ranged from 51 to 63 pounds for the 1933 spring wheat and from 49 to 63 pounds for the 1934 crop, while the range for the two crops of winter wheat combined was 55 to 63 pounds. The protein content of the 1933 spring wheat ranged from 10.00 to 18.50%, the 1934 spring wheat from 9.50 to 19.50%, and the winter wheat from 11.50 to 17.40%.

A 75% patent flour was made on an experimental mill by an experienced miller. A rather uniform extraction of flour was made, based on the test weight per bushel of wheat. The ash content of the flour was well under the 0.42% limit for patent flour.

¹ Contribution from Montana State College, Agricultural Experiment Station. Paper No. 93, Journal Series.

This method of securing material for the study of oven spring of dough in relation to certain properties of bread is believed to have been productive of flour having a wide range of strength and properties rather typical of Montana conditions.

Methods and Equipment

The Standard A. A. C. C. Baking Method³ as described in Cereal Laboratory Methods (1935) was used and the basic formula was employed with the following exceptions: Three percent sugar was used instead of 2.5%, and the dough was punched by passing it between canvas-covered sheeting rolls instead of folding by hand. The molding was also done with the sheeting rolls and the resulting strip of dough tightly rolled and folded for the pan. The baking pans used were the tall form.

Temperature control. The room temperature was kept within the limits of 23° to 26° C., and the dough when mixed had a temperature of approximately 30° C. This was accomplished by bringing the temperature of the flour to approximately 30° C. by placing it in the proofing cabinet over night and keeping the solutions at the following approximate temperatures before combining them in a beaker to add to the flour preparatory to mixing, *viz.*: sugar-salt solution, 40° C.; water, 40° C.; and yeast suspension, 30° C.

The dough was fermented and proofed at $30^{\circ} \pm 0.5^{\circ}$ C. in a cabinet with revolving shelves as described by Whitcomb (1934). The thermometers in this cabinet revolved with the shelves.

The baking was done at $230^{\circ} \pm 5^{\circ}$ C. in an oven with revolving shelves. A pan of water was kept in the oven.

Measuring oven spring. The oven spring was determined by measuring the height of the dough in the pan just before it was baked and again measuring the height of the resulting bread, the difference between these two readings being considered as oven spring. This was accomplished by placing the pan containing the dough, or the bread, on a base and sliding a projecting arm down to the topmost point, and reading the height in centimeters on the standard which supported the arm. This is illustrated in Figure 1.

The reliability which might be placed in the oven spring of dough when the same flour was used and the temperature and other conditions were held fairly constant was shown by Whitcomb (1936) in his study of the influence of sugar, salt, and yeast on oven spring. Based on the variation in oven spring of the check doughs which he baked, it was concluded that an accuracy of about 0.5 cm. might be expected.

Scoring the bread. The scoring of the bread was done in such a way as to provide suitable values for study and comparison. The

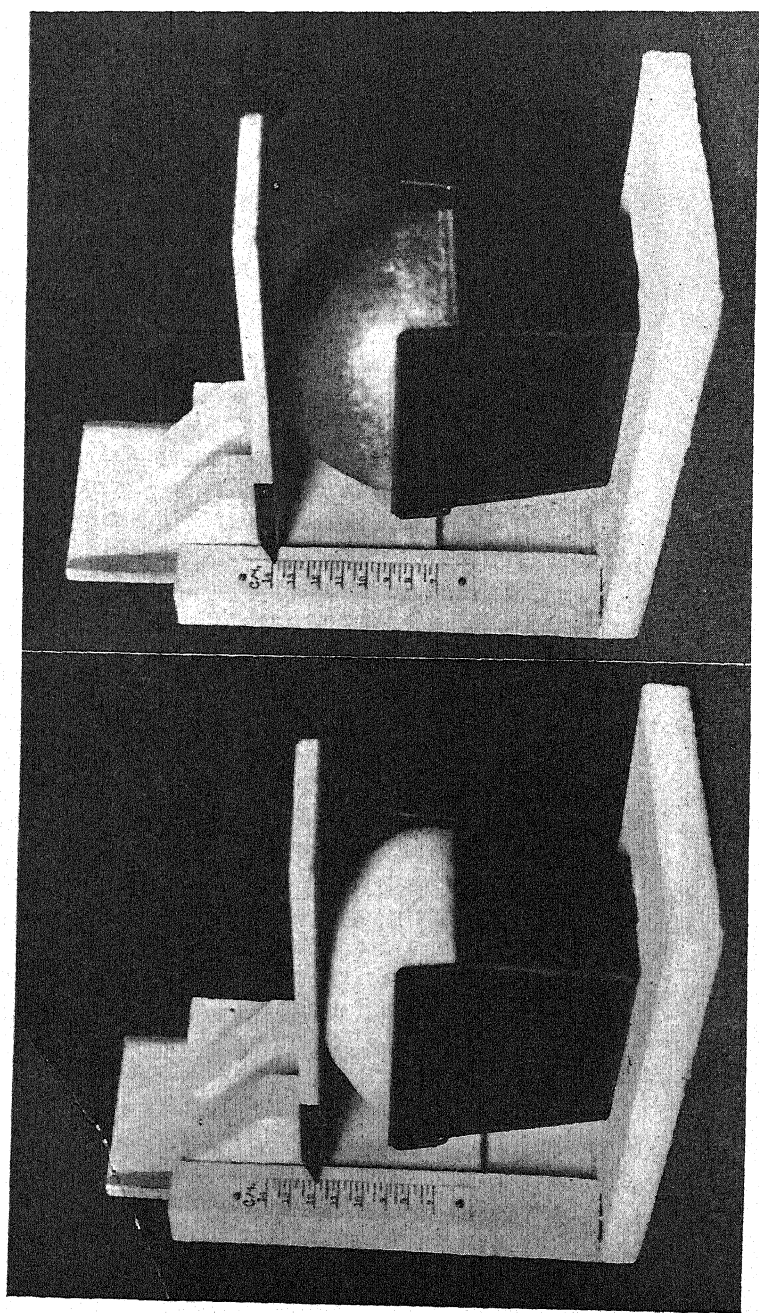


Figure 1. Oven spring measuring device. Left—Dough ready to place in oven. Right—Bread as it came from oven.

characteristics of crust color, boldness, and texture were scored on the basis of 100 as perfect. This standard was obtained by baking three check loaves from a good flour, made on a long system mill, with each day's baking. The color of crust was considered of importance as an indicator of the diastatic activity of the flour. However, a crust too dark in color was not considered desirable. Boldness of loaf is a term used to describe a loaf of well rounded appearance and one having a good shred and a lack of sagging at the ends and on the sides. The large loaves did not always have the best boldness. Boldness is referred to in the Standard Method as "symmetry." Texture took into consideration the size and shape of the cells of the cut surface of the bread and the thickness of the cell walls. The firmness of the bread was also a factor.

Results

The six characteristics of the wheat, flour, and bread which were studied in relation to the oven spring of the dough were loaf volume of bread, protein content of wheat, absorption of flour, color of crust of bread, boldness of loaf, and texture of bread. The statistical constants of mean, standard deviation, and coefficient of variability for the three lots of wheat under consideration are given in Tables I, II, and III.

TABLE I
STATISTICAL CONSTANTS FOR CERTAIN CHARACTERS OF WHEAT, FLOUR, AND BREAD—
HARD RED SPRING—1933 CROP

Characters	Mean	Standard deviation	Coefficient of variability
Oven spring of dough, cm.	1.14 ± .02	.56 ± .02	48.90 ± 1.79
Loaf volume of bread, cc.	561.50 ± 2.07	48.73 ± 1.46	8.68 ± .26
Protein content of wheat, %	14.84 ± .08	1.84 ± .06	12.40 ± .38
Absorption of flour, %	63.17 ± .11	2.51 ± .08	3.97 ± .12
Color of crust of bread, score	98.08 ± .09	1.99 ± .06	2.03 ± .06
Boldness of loaf of bread, score	97.72 ± .10	2.35 ± .07	2.42 ± .07
Texture of bread, score	98.53 ± .11	2.48 ± .08	2.52 ± .08

TABLE II
STATISTICAL CONSTANTS FOR CERTAIN CHARACTERS OF WHEAT, FLOUR, AND BREAD—
HARD RED SPRING—1934 CROP

Characters	Mean	Standard deviation	Coefficient of variability
Oven spring of dough, cm.	1.23 ± .03	.57 ± .02	46.25 ± 2.15
Loaf volume of bread, cc.	561.18 ± 3.80	69.05 ± 2.70	12.13 ± .48
Protein content of wheat, %	14.76 ± .14	2.48 ± .10	16.78 ± .66
Absorption of flour, %	61.09 ± .19	3.47 ± .13	5.67 ± .22
Color of crust of bread, score	97.47 ± .11	2.02 ± .08	2.07 ± .12
Boldness of loaf of bread, score	96.87 ± .17	3.08 ± .19	2.18 ± .12
Texture of bread, score	98.04 ± .15	2.71 ± .11	2.76 ± .11

TABLE III

STATISTICAL CONSTANTS FOR CERTAIN CHARACTERS OF WHEAT, FLOUR, AND BREAD—
HARD RED WINTER—1933 AND 1934 CROPS COMBINED

Characters	Mean	Standard deviation	Coefficient of variability
Oven spring of dough, cm.	.60 ± .02	.37 ± .02	61.73 ± 3.80
Loaf volume of bread, c.c.	510.18 ± 2.13	32.44 ± 1.50	6.36 ± .29
Protein content of wheat, %	13.83 ± .09	1.37 ± .64	9.91 ± .46
Absorption of flour, %	59.80 ± .28	4.29 ± .20	7.17 ± .33
Color of crust of bread, score	98.28 ± .08	1.26 ± .06	1.29 ± .06
Boldness of loaf of bread, score	93.73 ± .28	4.22 ± .20	4.51 ± .21
Texture of bread, score	95.64 ± .21	3.73 ± .17	3.90 ± .18

Variation of Characteristics as Shown by Statistical Constants

A comparison of characteristics of the wheat, flour, and bread which were studied shows some interesting relationships.

Oven spring. The oven spring of the dough, as measured in centimeters, had an average of 1.14 and 1.23 for the 1933 and 1934 crops of spring wheat and 0.60 for the two crops of winter wheat when combined. This shows the spring wheat flour capable of producing a dough having about twice the oven spring of that of winter wheat flour. The range of the oven spring was from 0.0 to 2.75 for the 1933 spring wheat, 0.0 to 2.50 for the 1934 spring wheat, and 0.0 to 1.75 for the winter wheat. The percentage of the 1933 spring wheat having no oven spring of dough was 2.8, that of the 1934 spring wheat 1.3, and that of the winter wheat 7.5. This shows a condition comparable with that of the mean oven spring when the spring and winter wheats are compared. The variability of the oven spring for the spring and winter wheats is also of significance. As shown by the standard deviation, two-thirds of the lots of 1933 spring wheat fell in the range of 0.58 to 1.70 centimeters oven spring, while for the 1934 spring wheat it was 0.66 to 1.80, and for the winter wheat it was 0.23 to 0.97.

Protein of wheat. The mean protein contents of the spring wheat were 14.84 and 14.76% for the two crops while for the winter wheat as combined it was 13.83%. The standard deviations for the protein content each side of the mean were 1.84 and 2.48 for the spring wheats and 1.37 for the winter wheat.

Loaf volume. The mean loaf volumes of bread in cubic centimeters for the spring wheats were 561.50 and 561.18, respectively, and for the winter wheat 510.18, with standard deviations above and below these of 48.73 and 69.05 for the spring wheat and 32.44 for the winter wheat.

Absorption of flour. The mean absorptions of the spring wheat flours were 63.17 and 61.09%, while that for the winter wheat flour

was 59.80%. The standard deviations above and below these were 2.51 and 3.47 for the spring wheat and 4.29 for the winter wheat.

Color of crust of bread. The mean scores for the color of crust for the spring wheat were 98.08 and 97.47, and for the winter wheat 98.28. The standard deviations above and below these were 1.99 and 2.02 for the spring wheats and 1.26 for the winter wheat.

Boldness of loaf. The mean scores for boldness of loaf for the spring wheat were 97.72 and 96.87, respectively, while for the winter wheat it was 93.73. The standard deviations above and below these means were 2.35, 3.08, and 4.22, respectively.

Texture of bread. The mean scores for texture of the bread from spring wheat flour were 98.53 and 98.04 for the two lots and 95.64 for that from the winter wheat flour. The standard deviations about these means were 2.48, 2.71, and 3.73.

The coefficients of variability of the various characteristics of these three lots of wheat and the resulting flours and breads as shown in Tables I, II, and III are of significance in showing their relative variability. With this as a measure, it will be noted that the oven spring of dough was the most variable, with protein content of wheat next. The loaf volume of bread appeared to be more variable in the spring wheat than in the winter wheat.

Correlation of Oven Spring of Dough with Certain Properties of Bread and Flour

Oven spring of dough as measured in centimeters was correlated with loaf volume of bread, protein content of wheat, absorption of flour, color of crust of bread, boldness of loaf of bread, and texture of bread. The results of these studies for three lots of wheat are given in Table IV.

The correlation of oven spring with loaf volume gave plus coefficients, the magnitudes of which were 0.53 for the 1933 spring wheat, 0.51 for the 1934 spring wheat and 0.50 for the 1933 and 1934 combined crops of winter wheat. The coefficients of correlation for oven spring and protein content of wheat were + 0.32 and + 0.27 for the two lots of spring and - 0.05 for the combined crops of winter wheat, thus showing a lesser degree of agreement. The absorption of the flour was not definitely associated with oven spring as shown by the correlation coefficients of - 0.36, + 0.35, and - 0.26. The crust color of bread was positively correlated with oven spring for the spring wheats, the coefficients being + 0.21 and + 0.22, while it was negative for the winter wheat, the coefficient being - 0.02. Boldness of loaf was definitely associated with the oven spring of the dough to much the same degree as the volume of the loaf, the coefficients being + 0.56

and + 0.57 for the spring wheats and + 0.55 for the winter wheat. The texture of bread was also definitely correlated with the oven spring of the dough, though with somewhat smaller coefficients, the magnitudes of which were + 0.40 and + 0.31 for the spring wheat and + 0.50 for the winter wheat.

As a matter of comparison, the loaf volume of bread was correlated with the protein content of the wheat. There was a definite positive correlation for the spring wheat, while there was a very small negative correlation for the winter wheat, the coefficients being + 0.66 and + 0.62 for the spring wheat and - 0.04 for the winter wheat.

TABLE IV

COEFFICIENTS FOR CORRELATION OF OVEN SPRING OF DOUGH WITH CERTAIN PROPERTIES OF BREAD, AND FOR LOAF VOLUME OF BREAD WITH PROTEIN CONTENT OF WHEAT, FOR THREE LOTS OF WHEAT

Properties correlated	Hard red spring wheat 1933 crop (254 tests)	Hard red spring wheat 1934 crop (152 tests)	Hard red winter wheat 1933 and 1934 crops (106 tests)
Oven spring of dough with loaf volume of bread	.53±.03	.51±.04	.50±.05
Oven spring of dough with protein content of wheat	.32±.04	.27±.05	-.05±.07
Oven spring of dough with absorption of flour	-.36±.04	.35±.05	-.26±.06
Oven spring of dough with color of crust of bread	.21±.04	.22±.05	-.02±.07
Oven spring of dough with boldness of loaf of bread	.56±.03	.57±.04	.55±.05
Oven spring of dough with texture of bread	.40±.04	.31±.05	.50±.05
Loaf volume of bread with protein content of wheat	.66±.02	.62±.03	-.04±.07

Discussion

The problem of associating certain properties of wheat and flour with those of the resulting bread has received attention by many investigators. The properties studied and the methods employed have varied considerably. The study of the oven spring of the dough in relation to certain properties of the bread and also to one property of the wheat and one of the flour is offered as a possible means of adding information of value. Dean and Swanson (1911) studied oven spring of dough in covered pans as influenced by fumigation of flour and concluded that it was not affected except in the low grade flour. Harrel (1926) studied the baking properties of flour as influenced by nine variables and noted that variation in fermentation period gave the most pronounced results in oven spring of dough. Whitcomb

(1936) studied the influence of sugar, salt, and yeast on the oven spring of dough with seven flours and concluded that both the flours and the ingredients had marked effects on the oven spring.

The variation of oven spring of dough, as shown in Tables I, II, and III, for flours from the spring and winter wheats would seem to have considerable significance when consideration is given to the baking properties of these two classes of wheat. The figures of 1.14 and 1.23 centimeters for the spring wheats as contrasted with 0.60 centimeters for the winter wheats are enough different to be of significance. It remains to be seen how this assumption works out when the oven spring of these two classes of wheat is correlated with some of the other properties of the bread.

As shown in Table IV, three of the correlation coefficients, in which oven spring is a part, for the winter wheat were negative and were of small order. These were when the factors of protein content of wheat, absorption of flour, and color of crust of bread were correlated with oven spring. These same coefficients for spring wheat were positive, except for absorption of flour for the 1933 crop of spring wheat which was -0.36 . On the other hand, the coefficients where oven spring was correlated with the factors of loaf volume, boldness of loaf, and texture of bread were all positive for both spring and winter wheat and were large enough to be of significance. A more detailed study of the correlations of oven spring with five of the properties of wheat, flour, and bread will be of interest.

*Loaf volume of bread.*² Perhaps more attention has been given to loaf volume and the factors affecting it than to any other property of bread baked in the experimental laboratory. The results of the present study, in which oven spring of dough is correlated with loaf volume and in which positive correlations of 0.50 or more have been obtained, compare favorably with those reported by other investigators. Blish and Sandstedt (1925) correlated loaf volume with viscosity of flour (unleached) and obtained a coefficient of $+0.245$. When they correlated loaf volume with protein content of wheat, a coefficient of $+0.304$ was obtained. Mangels (1926) studied correlation of loaf volume with protein content of hard red spring wheat for 11 crop years and reported coefficients ranging from -0.014 to $+0.547$. Hayes, Immer, and Bailey (1929) obtained correlations ranging from $+0.07$ to $+0.50$ for loaf volume and protein content of hard red spring wheat, and of -0.52 and $+0.11$ for hard red winter wheat. Bayfield (1934) studied baking properties of soft winter

² Note: In fairness to the investigators who published data on correlation of loaf volume with protein content prior to the adoption of the Standard A. A. C. C. Baking Test Method, it should be stated that better coefficients of correlation are to be expected with this improved method than with the old methods in use when the large loaf was baked.

wheats and reported correlations of loaf volume and protein of flour for the crop years of 1929 to 1932, ranging from $+0.4289$ to $+0.8247$. Later, Bayfield (1936) reported some correlation coefficients for protein of flour and loaf volume of bread in which tests were made on soft winter wheat flour both with and without bromate. For the 1931 crop, the coefficients were $+0.6397$ without bromate and $+0.7707$ with bromate. For the 1933 crop, the coefficients were $+0.6404$ without bromate and $+0.7991$ with bromate. These coefficients by Bayfield, even without bromate, which appear to be larger than those reported by other workers, may be explained by the fact that the wheat with which he worked may have had a higher diastatic activity, coming from a more humid region, than the wheat from the northwestern States. This also applies to the correlation coefficients for loaf volume and protein of wheat as reported in this study, which are $+0.66$ and $+0.62$ for spring wheat and -0.04 for winter wheat.

Protein content of wheat. This correlation of oven spring of dough with protein content of wheat gave coefficients of $+0.32$, $+0.27$, and -0.05 , which compare rather favorably with the earlier correlations as given above for loaf volume and protein but not with the later ones as reported by Bayfield; however, these coefficients are not considered large enough to be of significance in the present study.

Absorption of flour. Absorption of flour in relation to baking properties has apparently received limited attention. The correlations of -0.36 , $+0.35$, and -0.26 for oven spring of dough and absorption of flour are not considered of significance.

Color of crust of bread. It was thought when this work was planned that a good correlation might be secured between oven spring of dough and color of crust of bread, as both of these properties are probably associated with the diastatic activity of the flour. But such was not the case, as indicated by the small coefficients of $+0.21$ and $+0.22$ for the spring wheats and -0.02 for the winter wheat.

Boldness of loaf of bread. Although boldness of loaf of bread is not dependent upon the size of the loaf, slightly larger coefficients of correlation were secured between oven spring and this property than were obtained for oven spring and loaf volume. The coefficients in this case were $+0.56$ and $+0.57$ for the spring wheat and $+0.55$ for the winter wheat. A correlation between boldness of loaf and texture of bread might be expected to give interesting results.

Texture of bread. Texture of bread in relation to loaf volume has received consideration by several investigators, and this study of texture in relation to oven spring of dough seems to be of equal importance. Hayes, Immer, and Bailey (1929) correlated loaf volume and texture of bread for four crop years of hard red spring wheat

and obtained coefficients of $+0.07$, $+0.28$, -0.01 , and $+0.40$. The same correlations for two crop years of winter wheat gave coefficients of $+0.35$ and -0.03 . Treloar, Sherwood, and Bailey (1932) correlated texture and loaf volume for six crop years of hard red spring wheat and obtained coefficients ranging from $+0.096$ to $+0.329$, with an average of $+0.188$. In the light of the above results of correlation studies of loaf volume and texture of bread, the results of the present study in which oven spring was correlated with texture seem to be of special significance. These coefficients for oven spring and texture were $+0.40$ and $+0.31$ for spring wheat and $+0.50$ for winter wheat. This association of oven spring and texture would seem to indicate that there are a number of factors in the dough which determine the texture of the bread and that the property of oven spring is but one of these. At least, a further study of this phase of the problem should yield some interesting results.

Summary

The oven spring of dough in relation to certain properties of wheat, flour, and bread is offered as a possible aid in the study of strength of flour and quality of bread.

Studies were made on the relation of oven spring of dough to protein content of wheat, absorption of flour, and the four properties of bread—loaf volume, color of crust, boldness of loaf, and texture.

The material used was selected from the 1933 and 1934 crops of hard red spring and hard red winter wheats grown in Montana.

The oven spring of dough from spring wheat flour averaged twice as much as that from winter wheat, being 1.4 centimeters for the former and 0.6 centimeters for the latter.

Oven spring of dough, when correlated with loaf volume of bread, gave significant coefficients of $+0.52$ for spring wheat and $+0.50$ for winter wheat. This indicated that good oven spring of dough was an important function in the production of a loaf of bread of good volume.

Oven spring of dough, when correlated with protein content of wheat, gave insignificant coefficients of $+0.30$ for spring wheat and -0.05 for winter wheat. This would seem to indicate that oven spring of dough from the flours studied was not dependent upon amount of protein. Protein content of wheat was, however, significantly correlated with loaf volume of bread for spring wheat but not for winter wheat.

Oven spring of dough, when correlated with absorption of flour, and independently with color of crust of bread, gave insignificant coefficients for both spring and winter wheats.

Oven spring of dough, when correlated with boldness of loaf of bread, gave coefficients of about the same order of significance as for oven spring and loaf volume. This was true for both spring and winter wheats.

Oven spring of dough and texture of bread were significantly correlated for winter wheat and fairly so for spring wheat.

Literature Cited

- American Association of Cereal Chemists
1935 Cereal Laboratory Methods, Third Edition, 74-82.
- Bayfield, E. G.
1934 Soft winter wheat studies. II. Evaluating experimentally milled flours with the aid of viscosity, fermentation, and baking tests. Cereal. Chem. 11: 121-140.
- 1936 The influence of climate, soil, and fertilizers upon the quality of soft winter wheat. Ohio Agri. Exp. Sta. Bul. 563: 1-77.
- Blish, M. J., and Sandstedt, R. M.
1925 Viscosity studies with Nebraska wheat flours. Cereal Chem. 2: 191-201.
- Dean, George A., and Swanson, C. O.
1911 Effect of common mill fumigants on the baking qualities of flour. Kans. Agri. Exp. Sta. Bul. 178: 155-207.
- Harrel, C. G.
1926 Some variable factors of bread production. Cereal Chem. 3: 1-18.
- Hayes, H. K., Immer, F. R., and Bailey, C. H.
1929 Correlation studies with diverse strains of spring and winter wheats, with particular reference to inheritance of quality. Cereal Chem. 6: 85-96.
- Mangels, C. E.
1926 Relation of protein content to baking quality of flour from hard red spring and durum wheats. Cereal Chem. 3: 150-157.
- Treloar, Alan E., Sherwood, R. C., and Bailey, C. H.
1932 Some relationships involving crumb texture and color. Cereal Chem. 11: 121-127.
- Whitcomb, W. O.
1934 Temperature control in the baking test with revolving shelves in proofing cabinet. Cereal Chem. 11: 403-409.
- 1936 Oven spring of dough as influenced by sugar, salt, and yeast. Cereal Chem. 13: 698-702.

THE DETERMINATION OF SUGARS IN FLOUR

W. IWANOWSKI and C. GRABOWSKA

Institute of Technology of Fermentation and Foods, Polytechnical School,
Warsaw, Poland

(Received for publication April 6, 1937)

In actual practice the accurate analysis of flour and dough is attended with great difficulties, especially in connection with the determination of carbohydrates.

The determination of these substances, representing about 90% of the total dry material of flour, is of great importance, for it enables us to follow the changes taking place in the dough during processing and is indicative of the progress of starch hydrolysis.

Review of Present Methods for Determination of Sugars in Flour

The presence of enzymes and micro-organisms in flour and dough makes it difficult to determine sugars with accuracy. Consequently the methods that have been developed for this purpose are of necessity based on the inhibition of the activity of enzymes and micro-organisms.

One of the oldest analytical methods for the determination of sugars in cereal products is the alcohol method adopted by the A. O. A. C. (1935). With this method alcohol is used to inhibit the activity of the enzymes and lead acetate is used for clarification. This method is inconvenient, time-consuming and expensive, and its use makes it more difficult to determine dextrans in the presence of sugar. Many attempts have therefore been made to solve the problem of determining sugars in flour and dough by using other means of inhibiting the activity of enzymes and micro-organisms.

Extraction with water at 0° C. has been attempted (see Blish, Sandstedt, and Astleford, 1932) but diastatic action was not completely stopped. The same is true of extraction with water at 0° C. to which sulfuric acid has been added as in the Rumsey (1922) procedure. Hermano and Rask (1926) tried phenol and chloroform as inhibitors, and also experimented with water containing sodium tungstate, both at room temperature and at 1° C.

These methods are based upon the inhibition of enzymes and micro-organisms in an acid medium (pH lower than the optimum). It is questioned whether partial hydrolysis of sugar and starch may not take place after prolonged action by the acid.

Methods of stopping enzyme action with the aid of a pH greater than the optimum, *i.e.*, with the aid of alkali, have thus far not given positive results (see Blish, Sandstedt, and Astleford, 1932), possibly due to the destructive action of alkali upon sugar.

At the Institute of Technology of Fermentation and Foods of the Warsaw Polytechnical School work was begun on the determination of sugars in alkaline solution, using ammonia as an inhibitor. It was found, however, that ammonia was not satisfactory, because starch was dissolved and enzymic action was not wholly inhibited.

A 0.2 N solution of sodium carbonate was then tried as a means of inhibiting enzyme action. This solution, however, attacked sugar and starch, and the quantity of sugar found in the extract differed from that found in an alcoholic extract. Accordingly a suitable concentration of the solution was sought which would completely inhibit the action of the enzymes without attacking the sugar and starch.

To this end experiments were made to determine the effect of sodium carbonate solutions on sugar alone, on sugar in flour extracts, on malt, and on 14 previously analyzed wheat and rye flours.

Influence of Different Concentrations of Sodium Carbonate and Bicarbonate upon Sugar in a Water Extract

In studying the effect of the alkaline solutions on sugar, only maltose and glucose were taken into consideration. Saccharose was omitted, as it is known that this sugar is not hydrolyzed by alkali.

First of all the effect of 0.2 N solutions of Na_2CO_3 and NaHCO_3 on maltose and glucose, the fundamental sugars of flour and dough, was determined.

The addition of Na_2CO_3 or NaHCO_3 increased the pH of the solution used for extraction. Changes in the concentration of sugar were determined by means of the Bertrand (1906) copper reduction method, and were checked by simultaneous observations with a saccharimeter. Duplicate determinations were made, both at room temperature and at 6° C., and the experiments were repeated several times.

For these determinations there were prepared solutions of 1 gram of sugar (maltose or glucose) in (a) 100 c.c. of a 0.2 N Na_2CO_3 solution and (b) 100 c.c. of 0.2 N NaHCO_3 . Twenty cubic centimeters of these solutions were taken to determine the angle of rotation in a saccharimeter. After diluting the original solution in the proportion 1 to 5, 20 c.c. were used for determining reducing sugars by the Bertrand method.

The results of these experiments are recorded in Table I, the data there given being duplicate readings in each instance.

In these experiments with 0.2 N solutions of Na_2CO_3 and NaHCO_3 , variations were observed both in the number of cubic centimeters of KMnO_4 required to reduce 20 c.c. of the alkaline sugar solutions and in the angle of rotation obtained with the saccharimeter, for glucose as well as maltose. It was evident that these solutions acted on the sugar. The nature of these changes was not investigated.

TABLE I
EFFECT OF 0.2 N Na_2CO_3 —pH 10.48

Extraction time, hours	Temperature	Glucose				Maltose			
		Angle of rotation in degrees		C.c. KMnO_4 per 20 c.c. solution		Angle of rotation in degrees		C.c. KMnO_4 per 20 c.c. solution	
0	Room	3.3	3.3	7.71	7.71	7.5	7.5	3.81	3.81
5	"	2.9	2.9	7.59	7.59	7.2	7.2	3.85	3.85
10	"	2.8	2.8	7.58	7.58	7.0	7.0	3.80	3.80
0	"	3.1	3.1	8.29	8.30	7.5	7.5	4.38	4.40
5	"	3.0	2.9	8.39	8.39	7.3	7.3	4.31	4.31
20	"	2.9	2.9	8.40	8.40	7.2	7.3	4.25	4.25
0	"	3.0	3.0	8.18	8.15	7.5	7.5	3.80	3.82
5	"	3.0	3.0	8.20	8.20	7.5	7.5	3.95	3.95
10	"	2.9	2.9	7.76	7.78	7.5	7.5	3.85	3.85

EFFECT OF 0.2 N NaHCO_3 —pH 8.76									
0	Room	3.3	3.3	7.62	7.62	7.3	7.3	3.60	3.60
5	"	3.3	3.3	7.62	7.62	7.2	7.2	4.09	4.09
10	"	3.1	3.1	7.60	7.60	6.9	6.9	3.78	3.78
20	"	3.0	2.9	8.57	8.57	7.5	7.6	4.36	4.36
0	6° C.	3.1	3.1	8.50	8.50	7.7	7.7	4.45	4.45
5	"	3.0	3.05	8.54	8.54	7.6	7.6	4.40	4.40
10	"	2.9	2.95	6.20	6.20	7.3	6.28	3.15	3.15

Effect of 0.2 N Solutions of Na_2CO_3 and NaHCO_3 upon Sugar in a Flour Extract

The fact that both of these alkali solutions acted on both glucose and maltose in aqueous solutions indicated that the pH was too high. The next point investigated therefore was whether the natural acidity of flour would reduce the pH of an extract of flour made with 0.2 N Na_2CO_3 or NaHCO_3 to such an extent that sugar dissolved in the extract would not be decomposed. The following experiments were therefore made.

Ten grams of glucose or maltose were added to a suspension of 50 g. of flour in 1 liter of 0.2 N Na_2CO_3 or NaHCO_3 in a Stohman flask and the suspension shaken for 2 hours in a mechanical shaker, on the assumption that in this length of time the soluble components of the

flour would be completely extracted. Five grams of filtering asbestos (Schering-Kahlbaum No. 07773) were then added and the suspension filtered onto a Büchner funnel, using an S. & S. No. 572½ filter paper. The filtrate was clarified by adding 2 drops of basic lead acetate and a pinch of animal carbon (*Carbo animalis purissimus*, Merck, No. 2184E) and refiltering. By means of a pipette, 20 c.c. of the filtrate were transferred to a 100 c.c. volumetric flask and made up to the mark with 0.2 N Na_2CO_3 or NaHCO_3 . Portions of this solution were taken for the determination of sugar by the Bertrand method and for the measurement of the angle of rotation in the saccharimeter. The determinations made after 2 hours' shaking were taken as zero time. Similar suspensions were allowed to stand for 5 hours and 20 hours after shaking, and were then filtered and clarified in the manner just described. All these experiments were repeated three times. The results are shown in Table II. Figures for duplicate determinations are given.

TABLE II
EFFECT OF 0.2 N Na_2CO_3 AND NaHCO_3 UPON GLUCOSE AND MALTOSE
ADDED TO FLOUR EXTRACTS

Sugar	Time in hours	0.2 N Na_2CO_3 —pH 10.48				0.2 N NaHCO_3 —pH 8.76			
		C.c. KMnO_4 per 20 c.c. solution		Angle of rotation in degrees		C.c. KMnO_4 per 20 c.c. solution		Angle of rotation in degrees	
Glucose	0	6.70	6.70	2.4	2.4	6.70	6.70	2.6	2.6
"	5	6.75	6.75	2.3	2.3	6.90	6.90	2.2	2.2
"	20	6.75	6.75	2.3	2.3	6.93	6.93	2.2	2.2
Maltose	0	3.34	3.34	5.5	5.5	3.68	3.68	6.2	6.2
"	5	3.35	3.35	5.5	5.5	3.49	3.49	5.5	5.5
"	20	3.30	3.30	5.5	5.5	3.49	3.49	5.5	5.5

These experiments proved that the acidity of the flour did not reduce the pH of an alkaline extract sufficiently to prevent changes in the sugars. The changes continue, though at a considerably slower rate. It was observed that the changes that took place with increasing time were smaller with Na_2CO_3 than with NaHCO_3 . For purposes of orientation, the following figures are given on the pH of the various extracts containing glucose and maltose:

- (1) 0.2 N Na_2CO_3 flour extract + glucose..... pH 10.40
- (2) 0.2 N Na_2CO_3 flour extract + maltose..... pH 10.44
- (3) 0.2 N NaHCO_3 flour extract + glucose..... pH 8.59
- (4) 0.2 N NaHCO_3 flour extract + maltose..... pH 8.73

The above differences in pH may be caused by the action of the enzymes or by the destructive action of the alkaline solution on the sugars.

The 0.2 N Na_2CO_3 flour extract was examined for mannose, and the phenylhydrazone of mannose was found, indicating the presence of mannose, which is the result of the isomerization of glucose, this being the first stage of the action of the alkali. As stated in the foregoing, these alterations in the sugars may be brought about by too high a pH. In order to avoid this destructive influence, we used in our further experiments a mixture consisting of 75% of 0.2 N Na_2CO_3 and 25% of 0.2 N NaHCO_3 .

Experiments with this mixture were made by the same procedure as before, except that instead of animal carbon, "Celite" was used to decolorize the filtrate. This change was dictated by the belief that carbon absorbs a portion of the sugar, especially maltose, which has a relatively large molecule, whereas "Celite" is used in sugar refineries and has proved to be efficient.

The results obtained with the mixture of Na_2CO_3 and NaHCO_3 in flour extracts containing added sugar are shown in Table III. Figures for duplicate determinations are given.

TABLE III

EFFECT OF MIXTURE CONTAINING 75% 0.2 N Na_2CO_3 AND 25% 0.2 N NaHCO_3 UPON ADDED SUGARS IN FLOUR EXTRACTS

Sugar	Temperature	Time in hours	C.c. KMnO_4 per 20 c.c. solution		Angle of rotation in degrees	
Glucose	Room	0	6.55	6.55	1.9	1.9
"	"	5	6.55	6.55	1.9	1.9
"	"	20	6.65	6.65	1.9	1.9
"	6° C.	0	7.32	7.32	3.1	3.1
"	"	5	7.27	7.27	3.1	3.1
"	"	22	7.27	7.27	3.1	3.1
Maltose	Room	0	3.36	3.36	7.4	7.4
"	"	5	3.36	3.36	7.4	7.4
"	"	20	3.38	3.38	7.4	7.4
"	6° C.	0	3.99	3.99	7.45	7.45
"	"	5	3.85	3.85	7.45	7.45
"	"	20	3.85	3.85	7.45	7.45

The destructive effect of alkali on sugar was lessened by using the mixed soda solution. As changes occurred in the sugars during the first few hours, it was desirable to find out what happened to the maltose during the first two hours of shaking. In the previous experiments the first measurements were made after two hours of shaking the maltose with the soda solution and the flour. It was desired to determine whether the constancy of the values for maltose indicated that the mixed soda solution did not act on maltose or whether it was the result of equilibrium during the first two hours.

A series of experiments was made using the same method as before, and the changes that took place in the sugar after 0, 2 and 20 hours at room temperature were studied. Again differences were found in the sugar in the extract after various times, pointing to the fact that the quantity or quality had undergone change. The relative constancy of the results for maltose in the preceding experiments was therefore the result of equilibrium within the first two hours. The same can be said of glucose.

The differences observed may be the result of—

- (1) The action of enzymes
- (2) The action of micro-organisms
- (3) The destructive effect of the mixed soda solution on sugars.

Because of the high pH it does not seem likely that enzymatic action is a factor; the fact that the changes were observed chiefly during the first hours testifies against the supposed action of micro-organisms. Nevertheless it was decided to investigate the effect of an increase in concentration upon the values obtained.

For this purpose a solution of 75% of 0.3 N Na_2CO_3 and 25% of 0.3 N NaHCO_3 was used, under exactly the same conditions as in previous experiments. In all the experiments the differences in the values obtained increased for both glucose and maltose.

Thus the use of higher concentrations of the soda mixture was shown to be valueless, as it brings about considerable destruction of sugar.

Effect of Lower Concentrations of the Soda Mixture

Lower concentrations of the soda mixture were therefore tried, and a number of tests were run with different concentrations. It was found that a mixture of 75% of 0.15 N Na_2CO_3 and 25% NaHCO_3 was suitable for the purpose, as it does not change the composition of the sugars in an aqueous solution while it does inhibit enzymatic action.

The results of replicate tests are given in Table IV. The determinations made after 20 hours are omitted, as the earlier experiments showed no significant differences between the measurements after 5 and after 20 hours.

By using this concentration the maltose does not undergo any change that could be detected; on the other hand, considerable differences appear in the figures for glucose. One may assume that: (1) This concentration does not act on maltose but does attack glucose, (2) the enzymes are still active, and (3) the micro-organisms are still active. The second supposition may be rejected because the high pH excludes the possibility but the third is quite probable.

TABLE IV

EFFECT OF MIXTURE CONTAINING 75% 0.15 N Na_2CO_3 AND 25% 0.15 N NaHCO_3 UPON ADDED SUGARS IN FLOUR EXTRACTS

Sugar	Temperature	Time in hours	C.c. KMnO_4 per 20 c.c. of solution				Angle of rotation in degrees			
Glucose	Room	0	7.60	7.60	7.60	7.60	2.2	2.2	2.2	2.1
"	"	2	7.53	7.53	7.53	7.53	2.1	2.1	2.1	2.1
"	"	5	7.38	7.38	7.38	7.38	2.1	2.1	2.1	2.1
"	6° C.	0	7.60	7.60	7.60	—	2.2	2.1	—	—
"	"	2	7.38	7.38	7.38	—	2.1	2.1	—	—
"	"	5	7.38	7.38	7.38	—	2.1	2.1	—	—
Maltose	Room	0	4.00	4.00	4.00	4.00	6.3	6.3	6.3	6.3
"	"	2	4.00	4.00	4.00	4.00	6.3	6.3	6.3	6.3
"	"	5	4.00	4.00	4.00	4.00	6.3	6.3	6.3	6.3
"	6° C.	0	4.10	4.11	4.11	—	6.3	6.3	—	—
"	"	2	4.12	4.11	4.12	—	6.3	6.3	—	—
"	"	5	4.12	4.12	4.12	—	6.3	6.3	—	—

Effect of Adding Chloroform to the Soda Mixture

For this reason experiments were made in which chloroform was added in the process of shaking, as a means of checking the activity of micro-organisms. Our conjecture as to the action of micro-organisms proved to be correct. At room temperature the differences obtained were so insignificant that it would not be far wrong to say that the chloroform had checked the action of the micro-organisms. At the temperature of an icebox, viz., 6° C., the results obtained with the above concentration in the presence of chloroform were stable, from which it was inferred that the enzymatic and biological processes were completely checked.

The results of the experiments are shown in Table V. Replicate tests are given in each instance.

TABLE V

EFFECT OF ADDED CHLOROFORM UPON ACTION OF SODA MIXTURE (0.15 N) ON ADDED SUGARS IN FLOUR EXTRACTS

Sugar	Temperature	Time in hours	C.c. KMnO_4 per 20 c.c. of solution				Angle of rotation in degrees			
Glucose	Room	0	7.50	7.50	7.50	7.50	2.2	2.2	2.2	2.2
"	"	2	7.49	7.49	7.49	7.49	2.2	2.2	2.2	2.2
"	"	5	7.49	7.49	7.49	7.49	2.2	2.2	2.2	2.2
"	6° C.	0	7.30	7.30	7.30	7.30	2.2	2.2	2.2	2.2
"	"	2	7.28	7.30	7.30	7.30	2.2	2.2	2.1	2.2
"	"	5	7.30	7.30	7.30	7.30	2.2	2.2	2.2	2.2
Maltose	Room	0	4.00	4.00	4.00	4.01	6.3	6.4	6.3	6.4
"	"	2	4.00	4.00	4.00	4.00	6.4	6.4	6.4	6.4
"	"	5	3.98	3.98	3.98	3.98	6.4	6.4	6.4	6.4
"	6° C.	0	4.06	4.06	—	—	6.1	6.1	—	—
"	"	2	4.08	4.06	—	—	6.1	6.1	—	—
"	"	5	4.06	4.08	—	—	6.1	6.1	—	—

Proof is thus furnished that the mixture of 0.15 N solutions of Na_2CO_3 and NaHCO_3 with the addition of a few drops of chloroform is suitable for the determination of the sugars in flour, because it completely checks the action of enzymes and micro-organisms and at the same time does not attack sugar. The pH of the mixture itself was measured electrometrically and found to be 9.85.

Examination of Flour Extracts with the Refractometer

It remained to be settled whether this mixture did not attack starch and thus change the concentration of the extract. To this end experiments were made to determine whether the concentration of the flour extracts studied did not change with the course of time. At intervals of one hour, measurements were made of the refractive index of flour extracts by means of an Abbe refractometer. Under these conditions a change in the refraction could only be caused in consequence of the hydrolysis of starch, and would not be due to a change in the composition of the sugars already present in the solution.

The experiments were conducted as follows:

Fifty grams of flour were placed in a 1-liter Stolman flask and made up to the mark with a mixture of 75% 0.15 N Na_2CO_3 and 25% 0.15 N NaHCO_3 , to which a few drops of chloroform had been added. The contents were shaken for 2 hours. After adding filtering asbestos, part of the extract was taken for refractometric measurements and the balance was left on the flour and stored in an ice-box for later measurements. For purposes of comparison, parallel tests were made, using distilled water for extraction. One of the soda-mixture extracts was kept in an ice-box together with the water extract, while the others were kept at room temperature ($+20^\circ\text{C}$). The results of the experiments are shown in Tables VI and VII. Duplicate readings were made with each experiment.

To complete the data the refractive index of distilled water was determined and found to be 1.3321, and the refractive index of the soda mixture was found to be 1.3341.

The results set forth in Table VI show that no change in the refractive index occurs as the extraction time is increased, proving that the concentration of the extract does not change. It may therefore be concluded that the soda mixture used for the extraction of the flour does not disintegrate starch. As a further check on the previous experiment, more concentrated extracts were made up with the soda mixture, using 100 and 200 grams of flour per liter. It was found that regardless of the concentration, the refractive index of the extract did not change as the extraction time was increased.

TABLE VI
INFLUENCE OF 0.15 N SODA MIXTURE WITH CHLOROFORM UPON
REFRACTIVE INDEX OF FLOUR EXTRACTS

Type of flour	Time in hours	Index of refraction (duplicate readings)		Type of flour	Time in hours	Index of refraction (duplicate readings)	
Rye, 40% extrac- tion (Grasberg Mill, Warsaw)	0	1.3351	1.3351	Wheat, Stein- metz process	0	1.3344	1.3344
	1	1.3351	1.3351		1	1.3344	1.3344
	2	1.3351	1.3351		2	1.3344	1.3344
	3	1.3351	1.3351		3	1.3344	1.3344
	4	1.3351	1.3351		4	1.3344	1.3344
	5	1.3351	1.3351		5	1.3344	1.3344
	6	1.3351	1.3351		6	1.3344	1.3344
Rye, 55% extrac- tion, type 00/1. (Wichert Mill, Starograd)	0	1.3350	1.3350	Wheat, Perla Pomorska, G. L. 100	0	1.3328	1.3328
	1	1.3350	1.3350		1	1.3328	1.3328
	2	1.3350	1.3350		2	1.3328	1.3328
	3	1.3350	1.3350		3	1.3328	1.3328
	4	1.3350	1.3350		4	1.3328	1.3328
	5	1.3350	1.3350		5	1.3328	1.3328
	6	1.3350	1.3350		6	1.3328	1.3328
Rye, 65% extrac- tion, type 00 (Wichert Mill, Starograd)	0	1.3349	1.3349	Wheat (Wenus Steam Mill)	0	1.3346	1.3346
	1	1.3349	1.3349		1	1.3346	1.3346
	2	1.3349	1.3349		2	1.3346	1.3346
	3	1.3349	1.3349		3	1.3346	1.3346
	4	1.3349	1.3349		4	1.3346	1.3346
	5	1.3349	1.3349		5	1.3346	1.3346
	6	1.3349	1.3349		6	1.3346	1.3346
Rye, 40% extrac- tion, "Extra," (Torun Mill)	0	1.3352	1.3352	Wheat (War- saw Mill)	0	1.3346	1.3346
	1	1.3352	1.3352		1	1.3346	1.3346
	2	1.3352	1.3352		2	1.3346	1.3346
	3	1.3352	1.3352		3	1.3346	1.3346
	4	1.3352	1.3352		4	1.3346	1.3346
	5	1.3352	1.3352		5	1.3346	1.3346
	6	1.3352	1.3352		6	1.3346	1.3346
				Wheat, 60% extraction (Wichert Mill, Staro- grad)	0	1.3350	1.3350
					1	1.3350	1.3350
					2	1.3350	1.3350
					3	1.3350	1.3350
					4	1.3350	1.3350
					5	1.3350	1.3350
					6	1.3350	1.3350

As the figures of Table VII indicate, the index of refraction changes as the extraction time is increased when the flour is extracted with water, showing that the concentration of the extract has changed. At a temperature of 8° C. the change is less than at 30° C., because the enzymes are less active at the lower temperature. Increasing the concentration of the extract (Table VII A) by increasing the amount of flour up to 200 grams per liter resulted in a decided change in the values obtained.

In order to obtain more complete evidence as to the value of the soda-mixture extraction method for the determination of sugars in flour, several supplementary experiments were undertaken.

TABLE VII
REFRACTIVE INDEX OF WATER EXTRACTS OF FLOUR

Effect of temperature					
Type of flour	Temperature of extraction in ° C.	Time of extraction, hours	Grams of flour per liter	Index of refraction (duplicate readings)	
Rye, 40% extraction (Grasberg Mill, Warsaw)	8	0	50	1.3338	1.3338
		1		1.3341	1.3341
		2		1.3342	1.3342
		3		1.3342	1.3343
		4		1.3343	1.3343
		5		1.3343	1.3343
		6		1.3343	1.3344
Rye, 40% extraction (Grasberg Mill, Warsaw)	30	0	50	1.3336	1.3336
		1		1.3337	1.3337
		2		1.3334	1.3334
		3		1.3336	1.3336
		4		1.3340	1.3340
		5		1.3341	1.3341
		6		1.3344	1.3344
Rye, 40% extraction, "Extra," (Toruń Mill)	8	0	50	1.3339	1.3339
		1		1.3341	1.3341
		2		1.3341	1.3342
		3		1.3342	1.3342
		4		1.3342	1.3342
		5		1.3343	1.3342
Rye, 40% extraction, "Extra," (Toruń Mill)	30	0	50	1.3331	1.3331
		1		1.3334	1.3334
		2		1.3334	1.3334
		3		1.3335	1.3335
		4		1.3336	1.3336
		5		1.3336	1.3337

In a fermenting dough the enzymatic processes proceed at a rapid rate, and in order to carry out a determination of sugar one must work fast and stop the enzymatic action completely. In order to test out the method under extreme conditions, the mixture consisting of 75% of 0.15 N Na_2CO_3 and 25% of 0.15 N NaHCO_3 and containing a few drops of chloroform was allowed to act on malt for 5 hours, and the changes in the concentration of the extract with increasing extraction time were determined with the refractometer.

Fifty grams of dry malt in a Stohman flask were made up to the mark with the soda mixture and shaken for two hours in a mechanical shaker. After the addition of filtering asbestos, part of the extract was filtered off and its refractive index determined, while the remainder was held at 8° C. or 30° C. for further testing. For purposes of comparison parallel tests were run, using distilled water instead of the soda mixture. The results are given in duplicate in Table VIII.

TABLE VII A

Effect of concentration			
Type of flour	Time of extraction, hours	Grams of flour per liter	Index of refraction
Wheat, "Standard B," (Mill of Warsaw)	0	50	1.3330
	1		1.3330
	2		1.3330
	3		1.3332
	4		1.3345
	5		1.3345
Wheat, "Standard B," (Mill of Warsaw)	0	100	1.3338
	1		1.3339
	2		1.3339
	3		1.3345
	4		1.3338
	5		1.3338
Wheat, "Standard B," (Mill of Warsaw)	0	200	1.3337
	1		1.3338
	2		1.3339
	3		1.3348
	4		1.3355
	5		1.3355
	24		1.3357

TABLE VIII

REFRACTIVE INDEX OF MALT EXTRACT

Extraction medium	Temperature of extraction in ° C.	Time in hours	Index of refraction (Two sets of duplicate readings)			
Soda mixture	8	0	1.3359	1.3359	1.3360	1.3360
		1	1.3361	1.3361	1.3365	1.3365
		2	1.3361	1.3361	1.3368	1.3368
		3	1.3361	1.3361	1.3368	1.3368
		4	1.3361	1.3361	1.3369	1.3369
		5	1.3362	1.3362	1.3369	1.3369
Distilled water	8	0	1.3339	1.3339	1.3339	1.3339
		1	1.3345	1.3345	1.3344	1.3344
		2	1.3349	1.3349	1.3349	1.3349
		3	1.3350	1.3350	1.3349	1.3349
		4	1.3352	1.3352	1.3352	1.3352
		5	1.3352	1.3352	1.3352	1.3352
Distilled water	30	0	1.3341	1.3341	1.3340	1.3340
		1	1.3350	1.3350	1.3348	1.3348
		2	1.3352	1.3352	1.3349	1.3349
		3	1.3354	1.3354	1.3349	1.3349
		4	1.3355	1.3355	1.3350	1.3350
		5	1.3358	1.3358	1.3352	1.3352

A malt and flour extract, prepared from 10 grams of malt and 40 grams of 40% extraction rye flour (Grasberg Mill, Warsaw) in one liter of soda mixture, was also tested. The figures are given in Table IX.

The changes that occurred in the soda-mixture extract of the mixture of flour and malt are so small in comparison with those which occurred in the water extract that they can be considered as of no practical importance. The difference in the extracts after 4 or 5 hours suggests that in this case, due to the lowering of the pH by the acidity of the malt, the activity of the enzymes was not completely checked, but was slowed down to such an extent that no differences were noted during the first hours of extraction.

TABLE IX
REFRACTIVE INDEX OF EXTRACTS OF FLOUR AND MALT

Nature of extract	Temperature of extraction in ° C.	Time in hours	Index of refraction (duplicate readings)	
Soda mixture extract of rye flour and malt	8	0	1.3339	1.3339
		1	1.3340	1.3340
		2	1.3340	1.3340
		3	1.3340	1.3340
		4	1.3340	1.3340
		5	1.3341	1.3341
Distilled water extract of rye flour and malt	30	0	1.3339	1.3339
		1	1.3340	1.3340
		2	1.3340	1.3340
		3	1.3341	1.3341
		4	1.3343	1.3343
		5	1.3345	1.3345

Effect of the Soda Mixture with Chloroform upon the Activity of Micro-organisms

Having studied the effect of the mixture consisting of 75% of 0.15 N Na_2CO_3 and 25% of 0.15 N NaHCO_3 , with a few drops of chloroform, upon enzymatic activity, it remained to be determined how this mixture affected the viability of micro-organisms present in the flour extract.

As a nutritive medium for fungi, gelatine was used, made up with water and with soda-mixture extracts of malt and flour. A 10% solution of unhopped malt extract was used as a nutritive medium for bacteria.

The extracts were prepared from flour and malt by the above-described method, using 50 grams of flour or malt made up to one liter with soda mixture or water.

Seeding was carried out at dilutions of approximately 1, 1:10 and 1:100, designated respectively as I, II and III. The infected nutritive medium was poured into sterile Petri dishes 10 cm. in diameter. The dishes were placed in an incubator and examined after 24, 48 and 72 hours.

The data recorded in Table X show the numbers of colonies of molds, yeasts and bacteria that grew in various times and at different

TABLE X

GROWTH OF MOLDS, YEASTS AND BACTERIA ON SUBSTRATES PREPARED WITH WATER AND SODA-MIXTURE EXTRACTS OF FLOUR AND OF MALT

Extraction time, hours	Dilution	Soda-mixture extract of wheat flour			Water extract of wheat flour			Soda-mixture extract of malt			Water extract of malt		
		24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.
0	I	—	—	—	2	—	8	16	—	4	—	1 mold	Molded
0	II	—	—	1	—	Part liq. ¹	6 4 pl.	—	—	Liq.	—	Small colony	Great no. of col.
0	III	—	—	—	—	—	3 mold	—	—	1 mold	—	—	4
2	I	—	—	—	—	Part liq.	Liq.	—	—	—	—	12	Liq., great no. of col.
2	II	—	—	—	—	—	Liq.	—	—	—	—	Liq.	great no. of col.
2	III	—	—	—	—	4 mold	Liq.	—	—	—	—	—	32
4	I	—	—	3	—	10	Mold	—	—	—	—	Great no. of col.	great no. of col.
4	II	—	—	—	—	—	14	—	—	—	—	2	Liq., great no. of col.
4	III	—	—	—	—	Part liq.	5	—	—	—	—	2	Liq.
6	I	—	—	—	—	—	—	—	1 mold	3	—	—	—
6	II	—	—	—	—	—	—	—	—	—	—	15	—
6	III	—	—	—	—	—	—	—	—	—	—	—	—
24	I	—	—	—	—	—	3	—	—	—	—	—	—
24	II	—	—	—	—	—	11	—	—	—	—	—	—
24	III	—	—	—	—	—	1	—	—	—	—	—	—

¹ Liquefied.

dilutions on substrates prepared with water and soda-mixture extracts of flour and of malt. The minus sign indicates that no colonies were present.

These tests were repeated with four kinds of flour and two kinds of malt. A survey of all the data has led to the conclusion that a mixture consisting of 75% of 0.15 N Na_2CO_3 and 25% of 0.15 N NaHCO_3 , with a few drops of chloroform, completely checks the growth of

micro-organisms, as a substrate infected with a flour or malt extract prepared with such a mixture generally remains sterile, whereas on a substrate infected with a water extract the micro-organisms develop very readily. This confirms the findings of the previous experiments.

Electrometric Determinations of pH in Solutions and Extracts

In order to record complete information as to the conditions existing during the foregoing experiments, the pH of the solutions and extracts used was determined at the same time. The results of these measurements are given in Table XI.

TABLE XI
pH OF SOLUTIONS AND EXTRACTS USED IN THE EXPERIMENTS

	Potential, millivolts	pH
0.2 N Na_2CO_3	868.1	10.48
0.2 N NaHCO_3	768.3	8.76
0.2 N Na_2CO_3 extract of—		
Flour + glucose	863.8	10.40
Flour + maltose	866.4	10.44
0.2 N NaHCO_3 extract of—		
Flour + glucose	758.5	8.56
Flour + maltose	766.8	8.73
0.2 N soda-mixture extract of—		
Flour + glucose	855.1	10.25
Flour + maltose	857.1	10.29
0.3 N soda-mixture extract of—		
Flour + glucose	853.9	10.23
Flour + maltose	850.7	10.18
0.15 N soda-mixture extract of—		
Flour + glucose	850.0	10.16
Flour + maltose	851.8	10.20
0.15 N soda-mixture extract of—		
40% rye flour (Warsaw Mill)	850.0	10.16
55% rye flour (Wichert Mill)	846.2	10.10
65% rye flour (Wichert Mill)	847.4	10.12
Steinmetz flour	841.8	10.02
40% rye flour (Toruń Mill)	847.3	10.12
Rye flour (Frydrychewicz Mill)	844.6	10.07
Buffer mixture	653.6	6.81
(Equal parts of M/15 Na_2HPO_4 and M/15 KH_2PO_4)		

Conclusions

The experiments described in the foregoing have led to the conclusion that a mixture consisting of 75% of 0.15 N Na_2CO_3 and 25% of 0.15 N NaHCO_3 , containing a few drops of chloroform, is suitable as an extraction medium (at 6° C.) for the determination of sugars in flour. The concentrations of carbonates used do not destroy the sugars pre-existing in the flour, do not decompose the starch or protein materials, and completely check the activity of enzymes and micro-organisms. In comparison with other methods, this method is cheaper, simpler, and requires less time for extraction. It permits extraction

with an aqueous solution which is much cheaper than the alcohol used in other methods.

No other method of this nature has been described in the literature. In all the other methods the extractions are carried out in an acid medium.

Literature Cited

Association of Official Agricultural Chemists.

1935 Official and Tentative Methods of Analysis of the A. O. A. C., Fourth Edition (Sec. 28, p. 341). Published by the Association of Official Agricultural Chemists, Washington, D. C.

Bertrand, G.

1906 Le dosage des sucres reducteurs. *Bull. soc. chim. Paris* **35**: 1285.

Blish, M. J., Sandstedt, R. M., and Astleford, G. R.

1932 Sugars, diastatic activity, and "gassing power" in flour. *Cereal Chem.* **9**: 378-393.

Hermano, A. J., and Rask, O. S.

1926 A consideration of certain reactions of starches with special reference to enzyme hydrolysis. *Cereal Chem.* **3**: 361-392.

Rumsey, L. A.

1922 The diastatic enzymes of wheat flour and their relation to flour strength. *Am. Inst. Baking Bull.* **8**.

A COMPARISON OF THE LONG AND SHORT GASSING POWER METHODS¹

C. F. DAVIS

The Western Star Mill Co., Salina, Kansas

(Read at the Annual Meeting, May 1937)

In 1933 Sandstedt and Blish introduced the pressuremeter for measuring the gas-producing power of dough. This device offered many advantages for such tests but had the rather serious disadvantage of requiring 4 to 6 hours to obtain the best results. The recent modification introduced by Sandstedt (1937) gives pressuremeter values at 2 and 3 hours which are thought to identify more thoroughly important factors of gas production in dough. This report is a summary and comparison of results obtained by the long- and short-time pressuremeter methods on forty samples of commercial flour selected to offer a wide range of gas-production values.

The 6-hour pressuremeter figures were obtained by mixing 10 g. of flour and 10 c.c. of distilled water in which was suspended 0.3 g. of fresh yeast. For the 2-hour and 3-hour pressuremeter values, 10 g. of flour were mixed with 10 c.c. of distilled water in which were suspended .5 g. of fresh yeast and .5 g. of yeast activator.² The diastatic activity values as determined by the Blish and Sandstedt (1933) ferricyanide method were also obtained on these flours to extend the comparisons of the test values.

In Table I are tabulated the mean values of duplicate tests by each of the above methods. The precision of each method is indicated by expressing the average difference between duplicates as a percent of the range of values differentiating the flours. The degree of precision is nearly the same for all methods except the 3-2 hour pressuremeter values, which gave relatively low figures for differentiating values. Flour differentiation by the 2-hour pressuremeter method and the diastatic activity method was of the same order while the low differentiation established by the 3-2 hour method may be compensated for by the meaning of these values. The exact critical point in the rate of gas production was not determined on all flours, but close observation of the rate of gas production on a few high gas-producing flours indicated that the critical point had been reached before the 2-hour reading was made, and

¹ Subcommittee report, 1936-37 A. A. C. C. Committee on Methods of Analysis.

² Yeast activator supplied by R. M. Sandstedt.

TABLE I
A COMPARISON OF LONG AND SHORT TIME PRESSUREMETER VALUES AND DIASTATIC
ACTIVITY VALUE ON 40 SAMPLES OF COMMERCIAL FLOUR (ALL RESULTS
AVERAGE OF DUPLICATE TESTS)

Sample number	Pressuremeter values				Maltose per 10 g. flour
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	
	6 hour	2 hour	3 hour	3-2 hour	
	3% yeast	5% yeast and 5% activator			
	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mg.</i>
S-33	226	205	220	15	129
S-38	270	240	261	21	159
S-41	281	283	299	16	210
K-8	284	274	293	19	218
S-26	330	288	315	27	192
S-20	330	315	340	25	230
S-40	332	279	306	27	211
S-32	354	315	336	21	243
S-35	357	290	332	42	193
K-2	364	310	343	33	157
S-31	365	329	358	29	243
K-7	379	327	362	35	200
S-37	382	332	352	20	243
K-1	392	323	360	37	203
S-16	396	327	363	36	200
S-15	420	350	386	36	264
K-3	422	345	391	46	219
S-12	440	340	392	52	210
S-44	444	354	402	48	240
S-21	487	365	424	59	248
K-5	494	378	434	56	276
S-26	498	370	428	58	275
S-36	498	395	444	49	282
S-29	502	391	455	64	279
S-23	504	380	438	58	271
S-25	504	389	453	64	281
K-6	506	390	451	61	280
K-9	509	388	449	61	323
K-4	515	386	446	60	280
S-19	521	407	468	61	321
S-42	529	429	492	63	341
S-17	537	402	464	62	287
S-39	548	421	492	71	314
S-24	553	424	498	74	308
S-13	556	427	500	73	315
S-18	571	434	505	71	323
S-43	578	460	541	81	347
S-30	584	443	524	81	373
S-34	591	438	511	73	348
S-11	594	461	546	85	388
Mean	448.6	360.1	409.3	49.75	260.6
Mean difference between duplicates	3.30	3.2	3.4	2.25	3.27
Range of values	368	256	326	70	259
Duplicate differences as % of range	.9	1.2	1.0	3.2	1.3

$$R_{ab} + .983 \pm .004$$

$$R_{ed} + .865 \pm .027$$

$$R_{ac} + .988 \pm .004$$

$$R_{ad} + .967 \pm .007$$

$$R_{ae} + .902 \pm .020$$

the period from 2 hours to 3 hours was an index of the ability of the flour to sustain gas production after the readily available food was used up.

The correlation values between all gas production figures were sufficiently close to unity to indicate that the long- and short-time pressuremeter methods are measuring the same characteristics in flour. The diastatic activity values do not definitely place the flours in the order of their gas-producing ability as indicated by the correlation coefficients of $+ .902 \pm .020$ and $+ .865 \pm .027$. Some of the samples showing inconsistencies were tested for sucrose content and this factor accounted for the greater part of the variation, although other factors apparently entered into the difference in these test results. No further attempts were made in this study to account for these inconsistencies.

Summary

A comparison was made of the long and short gassing power methods and diastatic activity tests on forty commercial flours. The figures obtained were:

	Average value	Range of differentiating values	Average difference between duplicate tests	Duplicate difference as % of range
a. 6-hour pressuremeter values mm.	448.6	368	3.30	.9
b. 2-hour pressuremeter values mm.	360.1	256	3.20	1.2
c. 3-hour pressuremeter values mm.	409.3	326	3.40	1.0
d. 3-2 hour pressuremeter values mm.	49.75	70	2.25	3.2
e. Diastatic activity mg. maltose per 10 g. flour	260.6	259	3.27	1.3

$R_{ab} + .983 \pm .004$		Correlation coefficients	$R_{ad} + .967 \pm .027$	
$R_{ac} + .902 \pm .020$		$R_{ac} + .988 \pm .004$	$R_{de} + .865 \pm .027$	

Literature Cited

- Blish, M. J., and Sandstedt, R. M.
 1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* 10: 189-202.
 Sandstedt, R. M., and Blish, M. J.
 1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* 11: 368-383.
 Sandstedt, R. M.
 1938 A short gassing power method. Subcommittee report, 1936-37 A. A. C. C. Committee on Methods of Analysis. *Cereal Chem.* 15: 114-116.

AN IMPROVED WIDE-RANGE VOLUME-MEASURING APPARATUS FOR SMALL LOAVES ¹

D. S. BINNINGTON ² and W. F. GEDDES ^{3, 4}

Board of Grain Commissioners, Grain Research Laboratory, Winnipeg, Manitoba

(Read at the Annual Meeting, May 1937)

A volume-measuring device for small loaves was described by Geddes and Binnington (1928); this apparatus was later modified by Malloch and Cook (1930) and in this form is recommended by the A. A. C. C. (1935) to be used in conjunction with the Standard Baking Test. In either its original or modified form, it has been employed by the Canadian cereal laboratories co-operating with the Associate Committee on Grain Research of the National Research Council of Canada. In recent years the range and capacity of the apparatus were found inadequate and it was therefore decided to redesign it completely and investigate the whole question of loaf volume measurement with a view to ascertaining whether or not the present system could be improved.

Preliminary Considerations

Fundamentally, volumes of solids may be determined by three methods: (1) direct measurement and calculation, (2) density measurements, and (3) measurements of the displaced volume of either a gas, liquid or finely divided solid. The latter two methods are of course directly related but from a practical standpoint can be classed separately. As applied to the present problem, only displacement methods using either a liquid or a solid appear to possess any practical value, and the first of these is applicable only to research problems due to the necessity of water-proofing the loaf by impregnation with wax or some similar process. Under these circumstances, apparatus employing the displacement of a free-flowing solid offers the only feasible method of measuring loaf volume. The choice of a suitable solid is limited by the particular requirements involved, which may be summarized as:

¹ Contribution from the Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba. Published as Paper No. 126 of the Associate Committee on Grain Research of the National Research Council of Canada and the Dominion Department of Agriculture.

² Research Assistant, Associate Committee on Grain Research.

³ Chemist in Charge, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba.

⁴ The authors are indebted to J. G. Malloch, A. G. O. Whiteside, R. K. Larmour and A. G. McCalla for their valuable suggestions in connection with the design of this apparatus.

Free-flowing.

Minimum of adherence to the loaf and apparatus.

Uniformity of size and shape of particles, *i.e.* uniform packing characteristics.

Low density.

Unaffected by moisture.

Mechanical strength.

Minimum of abrasive action.

The only materials that appear to conform to the majority of the above requirements are various types of seeds, although small glass beads or metal shot would undoubtedly be superior were it not for their high density exerting a compressive effect upon the loaf. It is possible that some form of low density plastic might be developed for this purpose but nothing of this kind appears to be available at present. Of the various seeds proposed for this purpose, rape and flaxseed have been most widely used. Whitcomb (1925) carried out measurements of commercial-sized loaves in different types of apparatus employing rape, flaxseed, sweet clover, millet, vetch, wheat and peas and his data indicate that rape seed is probably the most suitable for small loaves.

The various mechanical devices used in conjunction with seed are all based essentially upon measurement of the volume of seed displaced by the loaf, although Whitcomb (1925) mentions a method employed by a German investigator in which the displaced seed is weighed and its volume computed. In its simplest form the seed displacement method is applied with the aid of a suitable box and large measuring cylinders. Apart from the time involved in making a test, this method is subject to serious error due to the fact that seed does not pack uniformly unless fed from a special distributing device. This "packing error" enters not only into the actual application of seed to the loaf but also to the measurement of both the original and residual seed volumes. An instrument in which seed is displaced upwards and around the loaf by means of a calibrated piston or similar device would eliminate this packing factor; experiments showed, however, that rape seed or flaxseed would not flow freely under such conditions and exerted a compressive effect upon the loaf. As apparatus of the hour-glass type helps to minimize the packing error, efforts were directed toward improving upon the earlier designs based upon this principle.

The normal working range of the original machine was 450 c.c. to 650 c.c.; this could be extended to 850 c.c. by allowing the seed to rise above the central diaphragm, or alternatively, a known volume of seed could be removed or a different size of standard loaf substituted. None of these alternatives is very satisfactory from a practical stand-

point and in any event the capacity is limited by the size of the measuring hopper which will not conveniently accommodate loaves in excess of 900 c.c. volume. With the instrument described by Malloch and Cook (1930) the normal working range was 550 c.c. to 950 c.c. and the measuring hopper was somewhat larger, but even with this improved design the apparatus was not capable of dealing with loaves of 1,000 c.c. to 1,300 c.c. volume, such as have been encountered in recent high protein years and by the use of new baking formulas. Obviously, the first step to be taken was to increase the size of the hoppers considerably but this alone does not solve the problem, because, unless the bore or length of the measuring tubes is materially increased, the normal working range remains at 400 c.c. Increased bore naturally means decreased accuracy and this is not desirable; on the other hand any worthwhile increase in length would render the apparatus cumbersome and fragile. The use of several standard loaves would accomplish the desired end but would be decidedly inconvenient, necessitating the addition or removal of seed and resetting of the zero point whenever loaves of a wide volume range were encountered.

Principles of New Design

In the new design these difficulties have been overcome and a continuous working range of 1,000 c.c. obtained by the following method:

One hopper is used as a seed receptacle only, the other serving for both calibration and measurement. Four identical lids are provided for this so-called loaf hopper, to one of which is permanently attached a metal standard loaf of exactly 900 c.c. volume; two of the remaining lids are fitted with solid rectangular metal "filler" blocks of 600 c.c. and 300 c.c. volume, respectively. The instrument is first standardized by fitting the "standard loaf lid" to the loaf hopper and adding seed to the zero mark on the graduated tube. The apparatus is then inverted, the seed transferred to the "storage" hopper, the "standard-loaf lid" removed and replaced by a measuring lid to which is attached the loaf whose volume is to be measured, the apparatus reinverted and a reading taken of the seed height in the tube. If the plain lid is used, loaf volume is obtained by adding 900 c.c. to the tube reading; with the 300 c.c. "filler-block" lid, by adding 600 c.c.; and with the 600 c.c. filler lid, by adding 300 c.c. It will thus be seen that the range of the instrument is as follows:

Plain lid	900 c.c. to 1,300 c.c.
300 c.c. "filler" lid	600 c.c. to 1,000 c.c.
600 c.c. "filler" lid	300 c.c. to 700 c.c.

Each lid has therefore a 100 c.c. overlap at each end of its range, making a critical matching of lid to loaf unnecessary, except in the case of occasional "borderline" samples. This method obviates the necessity of frequent interchange of standard loaves and resetting of the zero, thus enabling the baker to measure loaves to a five-minute schedule without assistance when baking up to 36 loaves a day.

Constructional Details

The general constructional details are illustrated in Figures 1 and 2 and the completed instrument in Figure 3. The seed-distributing

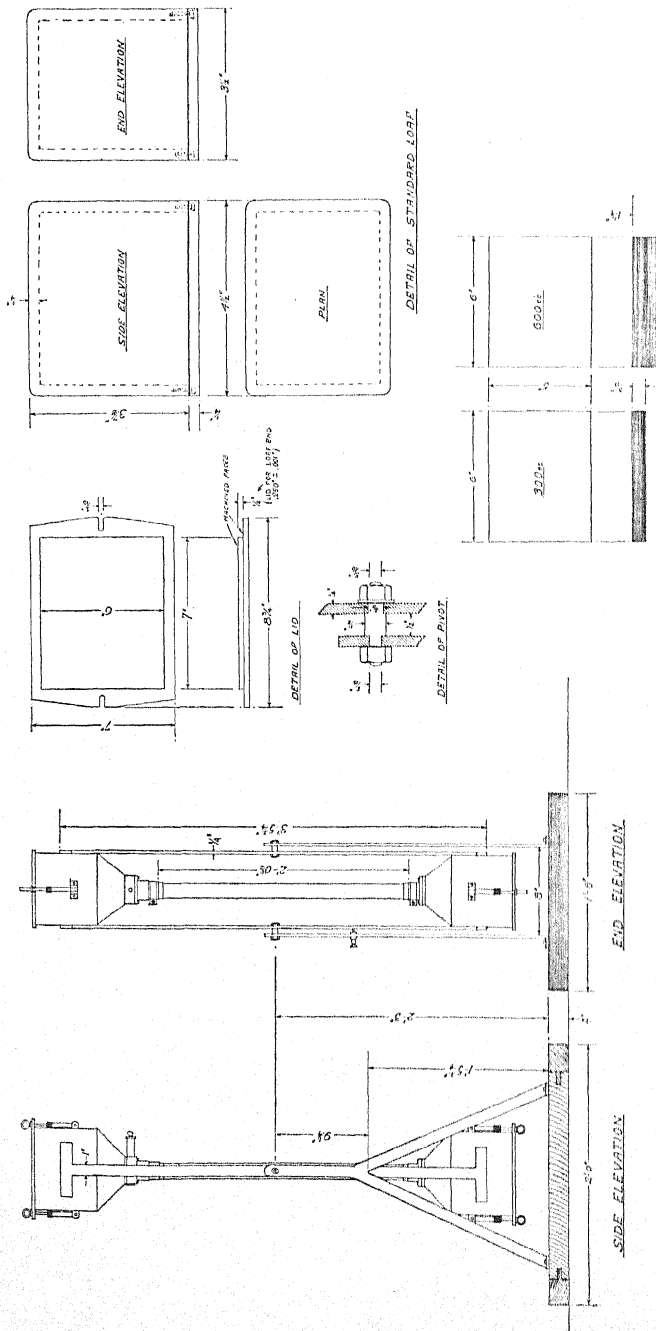


Figure 1. General constructional details.

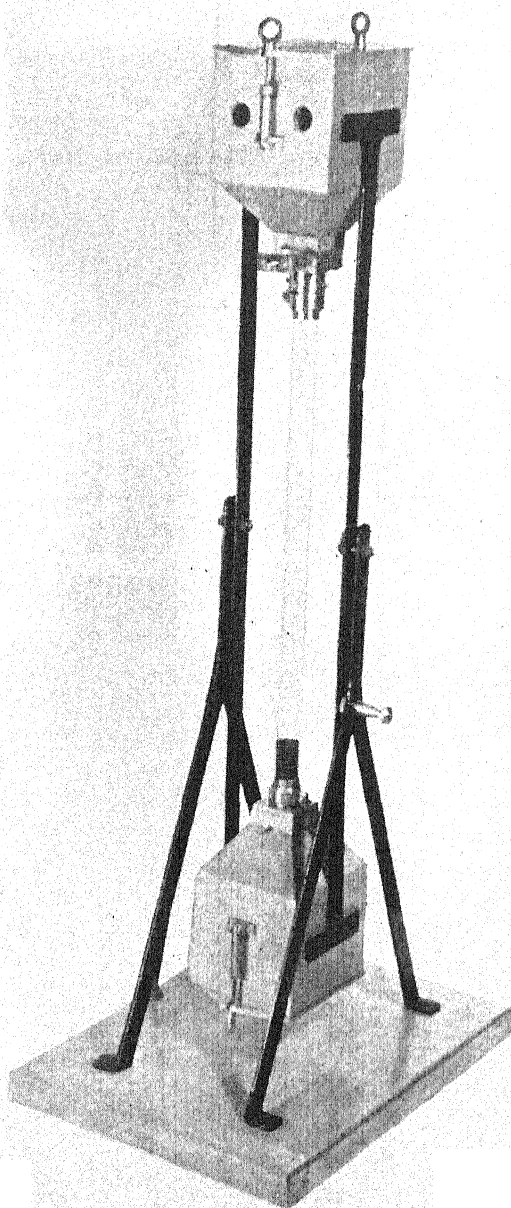


Figure 3. Loaf measuring device.

device described by Malloch and Cook (1930) has been employed with the slight modification of a double 60° hardened steel cone instead of the steel ball employed by these authors. This device provides a very uniform flow of seed and enables the full length of the measuring tube to be utilized. The general specifications are as follows:

Hoppers—Smooth, aluminum alloy castings internally free from roughness, ledges and sharp corners, and machined top and bottom.

Lids—Aluminum alloy, machined both sides, rebate on loaf lids to be 0.250 inch $\pm$ 0.001 inch. Rebate on seed hopper lid $\frac{1}{4}$ -inch (not critical). Minimum clearance between rebate and side walls of hopper. "Loaf-lids" to be fitted to hopper with square corners, "seed-lid" with round corners, in order to prevent interchangeability.

Filler blocks—Solid aluminum alloy, machined all over and adjusted to 600 c.c. and 300 c.c. volume $\pm$ 0.5 c.c. Volumes to be determined by weighing in air and water.

Standard loaf—Aluminum alloy, cast hollow, machined on bottom and closed watertight with machined aluminum plate. Final volume of 900 c.c. $\pm$ 0.5 c.c. to be obtained by machining and measurement as above.

Tube fittings—Cast brass, machined all over, caps for tubes to be fitted individually.

Sliding block—Aluminum alloy, machined all over, seed aperture exactly $\frac{3}{8}$ -inch in diameter. End plates of brass. A flat spring or similar device to be fitted in order to provide adequate friction and hold the slide in place.

Measuring tube—Of Pyrex glass, 35 mm. outside diameter, 25 inches long, graduated at 10 c.c. intervals, graduated portion to be centrally spaced. All lines to completely encircle the tube and each line numbered with as large figures as possible. Numbering to read *upwards*, i.e., zero at the bottom. Maximum allowable variation shall not exceed 1.0 c.c. on the total capacity.

Loaf attachment device—The three measuring lids shall be fitted with hardened steel spikes, as indicated, for impaling the loaf and in addition with suitable steel pegs or hooks whereby a rubber band may be stretched over the loaf to hold it in position. In the case of the 600 c.c. and 300 c.c. lids, these shall be short pins driven into the sides of the filler block. For the plain lid they shall be "L" shaped hooks fitted to the under surface of the lid.

General—The machine shall be so mounted as to pivot freely and be provided with a suitable spring-operated stop pin functioning at both ends of the travel.

Five machines were constructed according to the above specifications, the final machining and adjustment of the filler-blocks and standard loaves being made in the laboratory. The instruments were then assembled for test. As originally designed, the seed opening in the sliding block was only $\frac{1}{2}$ inch in diameter and under these conditions $4\frac{3}{4}$ minutes were required for complete transfer of seed. This rate was entirely too slow and the opening was enlarged by $1/64$ -inch increments until the satisfactory rate of $1\frac{3}{4}$ minutes was obtained with a $5/8$ -inch opening. During these tests it was noted that increasing the size of aperture and rate of feed caused the seed to pack more loosely and a volume increase of 130 c.c. was found between the two extremes. This clearly indicates the importance of an accurately controlled rate and manner of feed. At the final value, however ($5/8$ -inch and $1\frac{3}{4}$ minutes), the seed still packed so uniformly that even violent jarring of the apparatus caused no change in level.

Adjustment and Calibration

Following assembly of the machines and adjustment of the feed rate, the instruments were checked with four dummy loaves designated A, B, C and D, modelled from wood and waterproofed with shellac. Their true volumes were determined by weighing in air and water and loaves A, B and C measured on the "600 c.c." lid and loaves C and D on the "300 c.c." lid. For purposes of comparison, they were also measured by the conventional "box" method, together with one of the 900 c.c. metal standards. The results for a single machine are given in Table I.

TABLE I
TRUE AND APPARENT VOLUMES OF WOODEN DUMMY LOAVES

Loaf	Lid	True volume	Apparent volume, Machine No. 1	Volume difference true, Machine No. 1	Apparent volume, Box method	Volume difference true, Box method
		<i>C.c.</i>	<i>C.c.</i>	<i>C.c.</i>	<i>C.c.</i>	<i>C.c.</i>
A	600 c.c.	446	410	-36	403	-43
B	600 c.c.	545	500	-45	501	-44
C	600 c.c.	635	583	-52	574	-61
C	300 c.c.	635	588 (estimated)	-47		
D	300 c.c.	816	758	-58	753	-63
	900 c.c. Standard				843	-57

It will be noted that considerable discrepancies existed between the true and measured volumes, the instrument yielding low results in all cases, and furthermore that these differences were not consistent. These results were very disturbing and a careful check was accordingly made of all standardizations; in addition a critical analysis was made of possible sources of error from which it was finally concluded that the discrepancies were due to differences in seed packing caused by variations in both the size and shape of the loaf being measured. At first sight it would appear surprising that such effects had not been noted in early models of this type of machine. It is probable, however, that any such effect was minimized by the relatively small range and seed capacity of these earlier instruments. It was thought that the manner in which the seed struck the top of the loaf might be a controlling factor and one of the instruments was modified by the introduction of a blunt cone (35°) set immediately within the entrance to the measuring hopper. By this means the seed was thrown to the side and gradually filled up around the loaf instead of striking it directly. The use of this device increased the apparent volumes by from 46 c.c. to 93 c.c., thus making them from 10 to 35 c.c. "greater" than the actual volumes. Removing the cone but leaving the four

set screws in place with their points in contact gave apparent volumes differing from the true values by only -2 c.c. to $+13$ c.c. A series of tests was then made to determine the most satisfactory setting for these screws; this was found to correspond to a circular aperture of 16 mm. diameter, at which setting they were then locked firmly in place.

Although the introduction of these adjusting screws greatly reduced the error, discrepancies still existed when loaves of a considerable range in volume were measured. It therefore became necessary to construct calibration curves for each machine. A series of standards was essential for this purpose and in view of the apparently marked effect of size and shape of loaf upon the results obtained, it seemed highly desirable that such standards should closely approximate actual loaf shapes. This requirement precluded the use of water-filled rubber balloons as described by Harrel (1928) and employed by Heald (1929). Wooden dummies are tedious to construct, particularly if accurately modelled to prototype and aluminum models as described by Bailey (1930) require a pattern from which to be cast. As time was an important factor, models were prepared by baking an extensive series of loaves ranging in volume from approximately 300 c.c. to 1,500 c.c.; these were then thoroughly air dried and 12 loaves approximating the desired standards selected. Each loaf was trimmed to reasonably symmetrical shape with the aid of a rotating steel brush and a rasp, thoroughly impregnated with hot paraffin and built up to the desired shape and volume by modelling with dental wax. The final true volumes were obtained by weighing in air and water. The final true volumes to the nearest cubic centimeter were 315, 416, 506, 620, 662, 754, 867, 909, 956, 1045, 1124 and 1234 c.c., so that five to six loaves were available for checking each lid and filler block combination. With the aid of these wax models a correction chart was constructed for each machine and it is intended to use these dummy loaves as patterns for the production of permanent metal models.

Operation

In use, the loaf is impaled upon the spikes which are staggered in such a manner that the loaf may be easily cut for judging without interference from the indentations. A disc of thin dental rubber 2 inches in diameter is placed upon the top of the loaf and a rubber band of suitable tension employed to hold it firmly in position. This method of attachment is rapid and convenient to use and has no tendency to alter the loaf volume as is the case with spring clips applied to the ends of the loaf. The purpose of the dental rubber is to avoid the abrasive effect of the falling seed upon thin-crust or shell-top

loaves which, on account of the height of fall and quantity of seed rapidly cuts a hole in the top of such loaves. The zero point is checked with the standard loaf at frequent intervals (every 3 to 5 readings) and any deficiency in seed restored.

In order to secure an index of the precision of measurement, three of the wax dummy loaves were selected and 25 replicate readings made with each loaf on each of two successive days. The results of the uncorrected readings summarized in Table II show highly satisfactory replicability over the entire range of the apparatus:

TABLE II
PRECISION OF MEASUREMENT

Loaf model number..... Lid range in c.c.....	3 300 to 700		6 600 to 1,000		10 900 to 1,300	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Mean loaf volume, c.c.....	524	521	759	760	1049	1049
Minimum loaf volume, c.c.....	519	517	755	757	1046	1045
Maximum loaf volume, c.c.....	528	524	762	762	1052	1053
Standard deviation of loaf volume in c.c.....	2.60	1.94	1.74	1.32	2.17	2.37
Coefficient variation of loaf volume, %	0.50	0.37	0.23	0.17	0.21	0.23
Standard error of single determination (both days) c.c.....	2.60		1.54		2.25	

Effect of Shape and Surface Finish

Since it was found that both the size and shape of the object to be measured affected the results, it seemed desirable to attempt to secure an indication of the effect of loaf shape alone. Accordingly, a plaster mold was made from one of the wax models and a number of replicas cast in plaster of Paris. After thorough drying, three of these replicas were built up with the same weight of dental wax to represent (a) a normal, bold, symmetrical loaf, (b) a bold loaf with a "lop-sided" crown, and (c) a flat-topped, reasonably symmetrical loaf such as would be obtained from excessively runny doughs made from severely sprouted wheat flour. The true volumes of these loaves (ascertained by weighing in air and water) were (a) 888, (b) 893, and (c) 908 c.c., and the mean of ten uncorrected readings thereon 897, 911 and 948 c.c., respectively. Thus, the discrepancy between true and observed volume due to shape alone is 9, 18 and 40 c.c. for the "symmetrical," "lop-sided" and "flat-topped" loaves, respectively. This indicates that seed packing is definitely affected by loaf shape and that appreciable errors may occur in measuring abnormally shaped loaves.

In view of the desirability of constructing a set of permanent metal standards for calibration purposes, some experiments were conducted to determine whether the surface finish appreciably influenced the packing of the seed. To this end three of the plaster replicas previously referred to were water-proofed with several coats of lacquer. Thin aluminum foil was cemented to one; a second was provided with an exceedingly rough surface by applying a coat of nitro-cellulose cement and sprinkling with rough sand followed by a coat of lacquer to ensure adequate binding; the third was simply covered with a thin film of dental wax. The true volumes were then determined and ten replicate readings made with the measuring device. The corrected volumes differed from the true by -7 , $+7$ and $+2$ c.c. for the metal, rough and wax surfaces, respectively. While these differences are relatively slight, they indicate that a smooth, highly polished surface promotes closer packing of the seed as compared with a rough surface.

The use of plaster or metal models is open to the criticism that they are less resilient than bread and therefore the seed will rebound to a greater extent from their surfaces with a consequent effect upon the packing. That the use of the rubber disc upon which the seed strikes greatly minimizes this condition has been shown experimentally. These experiments suggest that in constructing permanent metal loaf models, the surface should preferably be not highly polished.

Discussion

The experiences encountered in the construction and calibration of these loaf-measuring devices indicate that such apparatus may be an important source of error within and between laboratories; for instance, the slightest variation in the position of the small set screws situated in the throat of the loaf-measuring hopper caused appreciable differences in apparent loaf volume and this emphasizes the sensitivity to slight variations in construction of any device based on the principle of seed displacement. That the size, shape and surface of the object to be measured introduced differences in the manner in which the seed packs has been definitely shown. When the volume of the system has been established by means of a rectangular shaped metal standard, a loaf-shaped object of the same volume will not give the same reading. However, in view of the difficulties involved in constructing loaf-shaped standards of metal, together with the fact that a calibration curve is essential in any event, the rectangular shaped standard as described is considered to be quite satisfactory for the purpose.

In view of the importance placed upon loaf volume and of present efforts of the Committee on Standardization of Laboratory Baking to

eliminate avoidable sources of variation, our experiences emphasize the need for a standard official apparatus, together with the provision of facilities for frequent recalibration.

Summary

The constructional details and specifications for a standardized loaf-measuring device based on the hour-glass principle employing rape seed and with a range of 300 to 1,300 c.c. are presented.

The apparent volumes obtained with such a device are influenced by the rate of seed flow and the manner in which the seed strikes the loaf, as well as by the size, shape, and surface finish of the object to be measured. It is consequently necessary to calibrate the apparatus with a series of loaf-shaped objects of known volume.

Loaf-volume apparatus may be an important source of error within and between laboratories and there is need for a standard official apparatus together with the provision of facilities for frequent recalibration.

Literature Cited

- American Association of Cereal Chemists
1935 Cereal Laboratory Methods, Third Edition. Published by the A.A.C.C.
Bailey, C. H.
1930 Calibration of loaf measuring devices with metal models. Cereal Chem. 7: 346-348.
Geddes, W. F., and Binnington, D. S.
1928 A volume measuring device for small loaves. Cereal Chem. 5: 215-220.
Harrel, C. G.
1928 Calibration of loaf volume boxes. Cereal Chem. 5: 220-222.
Heald, W. L.
1929 Calibration of loaf measuring apparatus. Cereal Chem. 6: 308-310.
Malloch, J. G., and Cook, W. H.
1930 A volume-measuring apparatus for small loaves. Cereal Chem. 7: 307-310.
Whitcomb, W. O.
1925 A study of methods of determining the loaf volume of bread. Cereal Chem. 2: 305-310.

TWO FURTHER SIMPLE OBJECTIVE TESTS FOR JUDGING CAKE QUALITY

VENONA W. SWARTZ

Department of Scientific Research, Institute of American Meat Packers,
Chicago, Illinois

(Received for publication September 13, 1937)

In addition to the excellent objective tests already reported in the literature, especially by Platt and Kratz,¹ the author has found two very simple additional objective tests helpful in evaluating cake quality. By the first test a measure can be obtained of the average relative size of the pores or grain of the cake. By the second, the ability of the cake to take up water is determined as one possible index to the eating characteristics of the cake. These tests will be described in detail, and some typical results given to show what may be expected.

Sand Retention Test for Grain of Cake

Photography gives a graphic record of the fineness or coarseness of the cake, but does not give results capable of quantitative comparison. For a quantitative test, some highly uniform substance, composed of small particles, if poured onto sections of the cake and allowed to penetrate the pores in a uniform manner and then weighed, should give a measure of the average size of the grain of the cake. Sand was selected for the substance to be used, because of its cheapness, its accessibility, its density, and its ease of sifting to uniform particle size. It was prepared for use by sifting through a 30-mesh sieve, and then using the portion retained by a 40-mesh sieve.

Four samples of each cake were prepared for the test by cutting horizontal sections one inch thick, free from top and bottom crust. The surface tested was the bottom of the slab, for the texture of the cake near the bottom is much more uniform than at the top. Rounds, two inches in diameter, were cut from these slabs with an ordinary biscuit cutter. These four pieces were then weighed individually to 0.01 g., as much care as possible being taken to prevent drying. All the pieces to be tested were covered very generously with the sifted sand, until no more would remain on them, and then each piece in turn was placed on a 40° incline and rotated once to permit as much sand to fall off as was not retained in the pores. The pieces were then

¹Platt, Washington, and Kratz, Philip D. Measuring and recording some characteristics of test sponge cakes. *Cereal Chem.* 10: 73-90 (1933).

weighed again, and the difference in weight recorded as a measure of the coarseness of the grain.

There are a number of factors that make for inaccuracy in this test. Listed briefly, they are: (1) Variation in texture of the cake from place to place and batch to batch; (2) variation in cutting of samples, *i.e.*, nearer or farther from bottom crust, surfaces not parallel, etc.; (3) variation in manner of adding sand; and (4) loss of weight by evaporation of moisture during test.

In spite of these factors, fairly consistent results can be obtained. Table I records some typical results on one cake, while Table II records the results that have been obtained on a series of yellow, white, and devil's food cakes made with lard and with a hydrogenated vegetable oil shortening.

TABLE I

ORIGINAL DATA ON SAND-RETENTION TEST FOR GRAIN. DEVIL'S FOOD CAKE MADE WITH LARD, FIFTH BAKING

Sample number	1	2	3	4
	G.	G.	G.	G.
Weight of cake + sand	18.71	19.75	19.22	20.61
Weight of cake	16.67	17.63	17.09	18.74
Weight of sand	2.04	2.12	2.13	1.87

TABLE II

SAND RETENTION OF YELLOW, WHITE, AND DEVIL'S FOOD CAKES MADE WITH LARD AND WITH HYDROGENATED VEGETABLE OIL SHORTENING
(Grams sand retained)

Baking number	Yellow ¹		White		Devil's Food	
	Lard	Hyd. V.O. ²	Lard	Hyd. V.O. ²	Lard	Hyd. V.O. ²
1	2.32	1.84	1.90	1.68	2.27	1.74
2	2.30	2.30	1.51	1.43	1.47	1.57
3	—	—	1.75	1.65	1.75	2.17
4	—	—	1.72	1.70	2.06	1.96
5	—	—	1.72	1.59	2.04	1.94
6	—	—	1.70	1.44	1.75	1.82
Average	2.31	2.07	1.72	1.58	1.89	1.87

¹ These data reprinted from "How to Use Lard in Making Bakers' Cakes," Baker's Helper, Chicago, May 15, 1937.

² Hydrogenated vegetable oil shortening.

The data presented in Table I show that at least a fair degree of agreement can be expected from four samples of the same cake.

The figures in Table II are indicative of the reliability of the test. In each pair of cakes, one made with lard and the other with the

hydrogenated shortening, the one made with lard had the more open grain, both according to inspection and according to the sand retention test. In the case of the three types of cakes, yellow, white, and devil's food, the difference between the cakes made with the two shortenings was least in the devil's food type of cake, both by inspection and by test.

Water Absorbing Ability, or "Wetability"

At least part of the eating character of cake is due to the ability of the cake to become moistened with saliva in the first few seconds of chewing. A cake that resists moistening will feel dry and unpleasant in the mouth, while one that moistens quickly is far more palatable. This quality or ability to absorb water is a simple measure also of the freshness of a cake, for the ability to take up water decreases as the cake becomes stale.

The test for this property is performed as follows: Four rounds of each cake being compared are cut exactly as for the sand-retention test, and the surface nearest the lower crust of the cake used. Each piece is weighed to 0.05 g. and placed into a Petri dish cover² containing 30 c.c. of water at room temperature. The cake is allowed to remain in the water exactly five seconds, removed, inverted quickly to prevent loss of water, and weighed again immediately. The difference in weight represents the water absorbing ability or the "wetability" of the cake.

Some typical results are given in Table III to show the agreement that may be expected in this test.

TABLE III
ORIGINAL DATA ON WATER-ABSORBING ABILITY. DEVIL'S FOOD CAKE MADE WITH LARD, SIXTH BAKING, THREE DAYS OLD

Sample number	1	2	3	4
	G.	G.	G.	G.
Weight of cake + water	28.50	25.45	27.00	27.75
Weight of cake	17.70	15.25	16.90	17.45
Weight of water	10.80	10.20	10.10	10.30

In Table IV results are given for three types of cakes, yellow, white, and devil's food, each made with lard and with a hydrogenated vegetable oil shortening.

These results show that cakes vary in their ability to take up water, and that as they become stale they lose that ability, some more rapidly than others. But since it is inherent in fresh cake, greater "wet-

² Diameter 9.7 cm. Depth of water approximately 5 mm.

TABLE IV

WATER-ABSORBING ABILITY OF YELLOW, WHITE, AND DEVIL'S FOOD CAKES MADE WITH LARD AND WITH HYDROGENATED VEGETABLE OIL SHORTENING

(Grams water absorbed)

Age in days	Yellow		White		Devil's Food	
	Lard	Hyd. V.O. ¹	Lard	Hyd. V.O. ¹	Lard	Hyd. V.O. ¹
0	13.59	13.65	14.58	10.37	12.72	10.32
1	12.61	12.71	12.95	7.07	11.68	8.25
2	11.73	11.98	12.20	6.25	10.81	7.27
3	11.53	11.82	11.71	5.26	9.82	6.30
4	10.98	11.80	11.69	4.72	9.48	5.60
5	—	—	11.01	4.26	9.17	5.21
7	—	—	10.10	3.51	8.51	4.32
Number of bakings	2	2	6	6	6	6

¹ Hydrogenated vegetable oil shortening.

ability" can be considered a desirable quality. While it is, of course, nothing more than an extremely simple index to eating quality, not taking into account flavor, aroma, velvetiness, color, tenderness, elasticity, general appearance, or any of the other important factors comprising eating quality, nevertheless a series of consumer acceptance tests has borne out its worth as an index. In tasting tests on the yellow cakes, over 1,000 individuals were practically equally divided as to preference for the two kinds. The "wetability" test shows the two yellow cakes very nearly equal in that quality. Of over 500 who tasted the white cakes, the cake made with lard was preferred by a ratio of 5 to 4. Again the preferred cake had the greater water-absorbing ability. The same relationship held true with the devil's food cakes. The lard cake was preferred in the ratio of 13 to 4 by 500 individuals, and as Table IV shows, its "wetability" was considerably greater than that of the cake with which it was compared.

Apparently, then, this ability of cakes to take up water rapidly is in some way correlated with a desirable eating quality. No other explanation is offered for this correlation except the observation that those cakes that have a poor "wetability" are the ones that feel dry in the mouth on eating.

A COMPARISON BETWEEN THE 100 AND 25 GRAM BAKING METHODS

R. H. HARRIS and T. SANDERSON

North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Received for publication September 16, 1937)

In evaluating new types of wheat produced by plant breeders it is of primary importance that a suitable and reliable test of quality be available which can be applied to a relatively small quantity of the wheat, thereby saving much valuable time to the breeder by ruling out as early as possible varieties which are quite unsuitable for the production of bread. The usual experimental milling and baking technique requires approximately 2000 g. of grain to furnish a complete picture of the milling and baking possibilities of the wheat. Several hundred grams of flour are necessary to obtain authentic information relative to the baking quality of the sample under examination in the instance of the customary baking procedure which calls for 100 g. of flour for each loaf. It is quite impossible to test out by this method small samples of flour which are often sent by mail for determination of baking quality. For these reasons other tests of quality have been persistently sought.

The wheat-meal fermentation time test was developed by Cutler and Worzella (1931, 1933) to solve this problem, while Pelshenke (1933) advocated a similar method for testing strength in native and imported German wheat. Bayfield (1935) concluded that the fermentation time test could only roughly group wheat on the basis of strength. These groups could be quite satisfactorily differentiated by the protein test and the time test could not be substituted for experimental milling and baking. Later Bayfield (1936) conducted an extensive investigation of the fermentation time test by comparing it with other well known criteria of baking quality and found that the baking procedure was the best test for flour strength. Bayfield and Shiple (1937) postulated that the Kjeldahl nitrogen determination is the most accurate and rigid single method of testing strength in soft winter wheat flours, but it does not take into account variations in protein quality. These workers found loaf volume and viscosity to be more valuable and accurate than the wheat-meal time test.

Geddes and Frisell (1935) described an experimental mill for producing flour from quantities of wheat as small as 100 g. Geddes

and Aitken (1935) published a description of an experimental baking technique designed to use only 25 g. of flour, and which could be satisfactorily employed with micro mill flour. Geddes and Sibbitt (1933) reported the findings of a preliminary study on this procedure and thought that it would differentiate satisfactorily between flours of different baking strength. Geddes and Aitken found the values obtained by the 25-gram technique to be highly correlated with the 100-gram loaf volume yielded by the same series of flour. This baking procedure appears to combine benefits obtained by the use of a relatively small sample with the possibility of obtaining information as to baking quality similar to that yielded by the Standard Baking Procedure, or its modification.

Material and Methods

In view of the advantages cited which accrue to the use of the micro baking method, it was decided to conduct a preliminary study of the relationship existing between the loaves produced by the new method and those obtained by using the 100-gram procedure using flour produced from North Dakota wheat. Accordingly, 76 samples of 70% patent flour experimentally milled from North Dakota hard red spring wheat were baked by the two methods. The doughs were mixed in the Hobart-Swanson for 2 minutes, 50 g. of flour being used in the instance of the small loaf and the doughs subsequently aliquoted before placing in the fermentation bowl. The baking tins employed were modeled after the ones used by Geddes and Aitken (*loc. cit.*) and were of the following dimensions: top 7.1×4.4 cm.; bottom 5.9×3.3 cm.; height 3.2 cm., similar in shape to the standard low-form tin. The loaves were measured in an apparatus of a rape seed displacement type. In other respects the baking formula followed was the Standard A. A. C. C. Procedure with 5% sucrose. The large loaves were baked in a similar manner (Standard Procedure plus 5% sucrose).

As Geddes has pointed out, the surface of the cut loaves from 25 g. of dough is too small to score satisfactorily for crumb color and texture, accordingly the only loaf characteristic considered here is loaf volume. This attribute is also subject to quantitative measurement.

Discussion

The loaf volumes obtained by the baking procedures are shown in Table I. The data are arranged in order of increasing wheat protein content, as this variable is commonly used in rating wheat strength. The 100-gram Standard Method did not give very large loaves even with the higher protein wheats of this series. Neither of the two baking methods used would appear to differentiate sharply among the

TABLE I
COMPARATIVE LOAF VOLUMES OBTAINED BY THREE BAKING METHODS

Sample number	Crude wheat protein (N×5.7)	Loaf volume	
		100 g. method.	25 g. method
		Standard basic	Standard basic
	%	<i>C.c.</i>	<i>C.c.</i>
1	13.8	413	90
2	14.5	469	108
3	14.6	402	95
4	14.9	417	96
5	15.1	403	96
6	15.4	460	126
7	15.4	470	114
8	15.4	453	101
9	15.6	442	98
10	15.7	386	107
11	15.8	465	109
12	15.9	362	110
13	16.1	472	113
14	16.3	319	85
15	16.3	386	91
16	16.3	397	98
17	16.4	458	97
18	16.4	319	79
19	16.4	487	106
20	16.4	455	96
21	16.4	494	123
22	16.5	442	101
23	16.5	374	87
24	16.5	392	94
25	16.5	400	108
26	16.5	515	125
27	16.6	376	96
28	16.6	376	95
29	16.6	417	105
30	16.7	433	97
31	16.8	470	110
32	16.8	497	108
33	16.8	500	114
34	16.8	391	91
35	16.8	487	134
36	16.8	533	133
37	16.9	524	110
38	16.9	504	118
39	16.9	475	101
40	16.9	356	89
41	17.0	467	117
42	17.0	478	117
43	17.1	406	104
44	17.1	545	130
45	17.1	454	101
46	17.1	597	136
47	17.1	342	92
48	17.2	534	123
49	17.2	457	97
50	17.2	585	112
51	17.2	471	132
52	17.2	480	115

TABLE I—*Continued*

Sample number	Crude wheat protein (N×5.7)	Loaf volume	
		100 g. method	25 g. method
		Standard basic	Standard basic
	%	C.C.	C.c.
53	17.3	569	133
54	17.4	563	137
55	17.4	564	117
56	17.4	560	135
57	17.4	558	134
58	17.5	542	120
59	17.5	543	119
60	17.5	551	130
61	17.5	577	111
62	17.5	538	121
63	17.5	554	131
64	17.8	574	108
65	17.8	567	120
66	17.8	563	120
67	17.8	590	130
68	18.0	532	127
69	18.0	582	118
70	18.1	515	117
71	18.1	567	122
72	18.2	507	106
73	18.3	610	123
74	18.5	616	132
75	18.6	575	147
76	19.5	475	96

wheats on a protein content basis. This is in agreement with a former paper (Harris, 1937), when no significant correlation between crude flour protein and loaf volume was found in the instance of 30 samples of 1936 North Dakota hard red spring wheat.

In Table II the statistical constants calculated from the baking data are shown. The Standard 100-gram Baking Procedure yielded the largest mean loaf volume and showed the greatest variability, while the 25-gram method had the least. The correlation coefficients show a significant relationship between wheat protein and loaf volume for the two methods.

While the relationship between the 100-gram and 25-gram loaves may not be of sufficient magnitude in the present instance to predict 100-gram loaf volume with great accuracy from a knowledge of the 25-gram loaf size, yet it is evident that undesirable and weak wheat varieties can, from the information made available by this procedure, be eliminated very much earlier than would be true otherwise, and much useless work thereby saved. Strong wheats, on the other hand, would be apparent also and their production speeded up with appreciable economy of time and effort.

TABLE II
TABLE OF STATISTICAL CONSTANTS
Mean Loaf Volumes, Standard Deviations, and Coefficients of Variability

	Means	Standard deviation	Coefficient of variability
Standard 100-gram procedure	481.6	74.88	15.53
Standard 25-gram procedure	111.6	15.07	13.50

Simple Correlation Coefficients

Variables correlated		r_{xy}	P^1
x	y		
Crude wheat protein	Loaf volume standard 100-gram bake	+.5918	<.0000002
Crude wheat protein	Loaf volume standard 25-gram bake	+.4566	.0000008
Loaf volume standard 100-gram bake	Loaf volume standard 25-gram bake	+.8068	<.0000002

¹ P = the probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.

Summary

A series of 76 experimentally milled North Dakota hard red spring wheat flours were baked by two procedures—the Standard 100-gram Method, using the basic ingredients of flour, water, sugar, salt and yeast, and a 25-gram procedure similar to the Standard Method described, except for quantity of flour used.

A significant correlation between loaf volume and wheat protein content was shown by the two baking methods. A relatively high positive correlation was found between the two Standard Methods. This relationship, while not sufficiently high to permit of accurately predicting 100-gram loaf volume, would prove useful in differentiating strong and weak wheat varieties, thereby justifying its use as a method for baking when sufficient material is not available for the Standard 100-gram Procedure.

Literature Cited

- Bayfield, E. G.
 1935 Observations on the whole wheat-meal fermentation time test. *J. Am. Soc. Agron.* 27: 241-250.
 1936 A collaborative study on the use of the wheat meal "time" test with hard and soft wheats. *Cereal Chem.* 13: 91-103.
 — and Shiple, V.
 1937 Soft winter wheat studies. V. Evaluating the quality and the strength of some varieties. *Cereal Chem.* 14: 551-577.
 Cutler, G. H., and Worzella, W. S.
 1931 A modification of the Saunders' Test for measuring "quality" of wheats for different purposes. *J. Am. Soc. Agron.* 23: 1000-1009.
 1933 The wheat-meal fermentation time test of "quality" in wheat as adapted for small plant breeding samples. *Cereal Chem.* 10: 250-262.

Geddes, W. F., and Aitken, T. R.

1935 An experimental milling and baking technique requiring 100 grams wheat. *Cereal Chem.* 12: 696-707.

— and Frisell, B.

1935 An experimental flour mill for 100-gram wheat samples. *Cereal Chem.* 12: 691-695.

— and Sibbitt, L. D.

1933 Variability in experimental baking. IV. Studies on mixing, sheeting rolls, pan shape and 50 and 25-gram formulas. *Cereal Chem.* 10: 560-584.

Harris, R. H.

1938 A study of gluten protein fractionation from sodium salicylate solution. Part II. Bread wheat gluten fractionation. *Cereal Chem.* 15: 80-90.

Pelshenke, P.

1933 A short method for the determination of gluten quality of wheat. *Cereal Chem.* 10: 90-96.

BOOK REVIEW

Wheat and Flour Quality. By C. O. Swanson. Published by Burgess Publishing Co., Minneapolis, Minn. 227 pages mimeographed. 1938. Price \$3.00.

The twenty-six chapters of this book were based upon the lectures delivered by Prof. Swanson to his classes at the Kansas State College during recent years. A variety of topics is covered, including definition and measurements of wheat and flour quality, constituents of wheat, types of wheat and the influence of soil and climate on quality, storage of wheat and flour, chemical technology of milling, and related subjects. Sections dealing with the statistics of wheat production and the position of bread in the diet are also included. Incidentally, the preparation of this manuscript afforded Prof. Swanson an opportunity to bring together in one book the material which had heretofore been scattered through several trade journals, and hence none too available to the average student of this subject.

The book is replete with useful facts. Sometimes related facts appear to be widely separated, as, for example, the data of pH, and the observations on diastatic activity. That may be the consequence of a system of lecturing, however, since often the sequence of presentation in a series of lectures is not perfectly adapted to monographic presentation. Discussion of the amylases is rather brief, in view of the prominence of these enzymes in present milling and baking practices, and the student of the subject might appreciate more expansive treatment than is accorded them here.

Such a large scope of subject matter is difficult of condensation within the confines of a book of this type. Dr. Swanson has made an excellent choice of material and one suspects that his greatest problem as an author involved choosing what to omit. Some of us would have been glad had he made the volume twice as large.

In view of Dr. Swanson's interest in mechanical testing of dough, the last two chapters in this volume entitled: XXV. Some factors at the basis of the physical property of dough, and XXVI. Physical tests to measure quality, will be of particular interest to those who have followed his work in this field. The author's recording dough mixer is clearly depicted, and an analysis of curves recorded by this device is presented. Earlier in the book (p. 37) curves are presented resulting from tests of a few characteristic varieties or classes of American wheats.

The time was ripe for a new book in this field written in the English language. Dr. Swanson has supplied certain needs admirably and his contribution will be welcomed.

C. H. BAILEY

CEREAL CHEMISTRY

VOL. XV

MAY, 1938

No 3

OATS AND OAT PRODUCTS

CULTURE, BOTANY, SEED STRUCTURE, MILLING, COMPOSITION, AND USES

HAROLD J. BROWNLEE¹ and FRANK L. GUNDERSON^{2,3}

The Quaker Oats Company, Chicago, Illinois

(Read at the Annual Meeting, May 1937)

Oats are a widely grown crop which provide important nourishment both as human food and as livestock feed in many countries. The oat plant is a prominent member of the cereal grass family and bears the botanical name *Gramineae Avena sativa*. The treatise entitled "L'Avoine" by the Frenchmen Denaiffe and Sirodot (1902) seems to be the most comprehensive publication on record devoted exclusively to oats. In the introductory part of their book of more than 800 pages oats are named in almost a score of languages, representing countries where the crop had importance at the beginning of the twentieth century. The respective names follow:

Allemand—Hafer; Anglais—Oat; Breton—Kerch; Danois—Havre; Espagnol—Avena; Flamand—Haver; Hollandais—Haver; Hongrois—Zab; Italien—Vena; Japonais—Enbaku; Latin—Avena; Norwegian—Havre; Polonais—Owies; Portugais—Veia; Roumain—Ovesul; Russe—Ovesu; Serbe—Ovas; Suedois—Hafre; Turc—Ionlaf.

Records indicate that the oat plant was originally observed more than forty centuries ago and that during the Bronze Age, 1500–500 B.C., it was first developed as a domestic crop. By the beginning of the seventeenth century the crop was extensively established in Western Europe. At that time, 1602, oat seeds are said to have been transported across the Atlantic Ocean to be planted for the first time in North America—in the New England States. Their cultivation in this country expanded to the point that George Washington is said to have sown on his plantation at Mt. Vernon, Virginia, in 1787 almost 400 acres of oats. From those early American colonial days oats have

¹ Formerly of The Quaker Oats Company, Cedar Rapids, Iowa; now with Quaker Oats Ltd., Southall, Middlesex, England.

² The Quaker Oats Company, Research Laboratories, Chicago, Illinois.

³ The authors are grateful for the cooperation of A. L. Winton, L. C. Burnett, and T. R. Stanton. They are also indebted to F. E. Raymond for his help in preparing the manuscript.

become progressively more important as a feed and food crop in the United States, being grown particularly in the northern States and consumed in all sections.

Culture of Oats

Two generations ago the American production of oats occurred mainly in the States of Ohio, Pennsylvania and New York. Gradually, with the settlement of newer areas, the culture of oats moved westward and northward until the chief oats-producing section now coincides rather closely with the so-called north-central "corn belt." One important difference, however, is that the oats area extends farther north than does that of corn. In terms of millions of bushels (32 pounds per bushel) produced in 1935, the foremost production figures for individual States were: Iowa 205, Minnesota 181, Illinois 107, and Wisconsin 85.

Oats fit well into the rotation scheme now prevalent in the diversified farming areas, being often grown between corn and wheat or between corn and grass or legumes.

Table I shows data taken from the Statistical Supplement of the latest United States Department of Agriculture Yearbook of Agriculture (1936a). According to the figures, oat production reached its peak about the year 1915 with a volume of 1,435,270,000 bushels.

TABLE I
OATS ACREAGE, PRODUCTION, AND VALUES IN U. S. A. AT DECENNIAL INTERVALS

Year	Acreage harvested (1000 acres)	Production (1000 bu.)	Farm value (1000 dollars)
1875	13,616	364,967	134,088
1885	23,351	674,151	187,860
1895	30,905	924,858	178,767
1905	33,426	1,104,395	317,843
1915	38,802	1,435,270	550,065
1925	44,240	1,410,336	547,231
1935	39,714	1,195,435	333,960

In terms of domestic tonnage produced in 1935 the three leading cereal grains named in order of decreasing volume are corn (maize), oats, and wheat. If one arbitrarily assigns corn tonnage the value of 100, then the comparative figure for oats is 35, and for wheat it is 33. The severe drouth which has prevailed through the grain belt during the last six or seven years is responsible in part for the lower 1935 production of corn, oats, and wheat.

Botany of Oats

The botanical classification of the oat plant, *Gramineae Avena sativa*, is summarized very thoroughly by Stanton (1936) of the United

States Bureau of Plant Industry, who has kindly permitted reproduction of his excellent photograph of the heads of several stalks of oat plants (Figure 1).

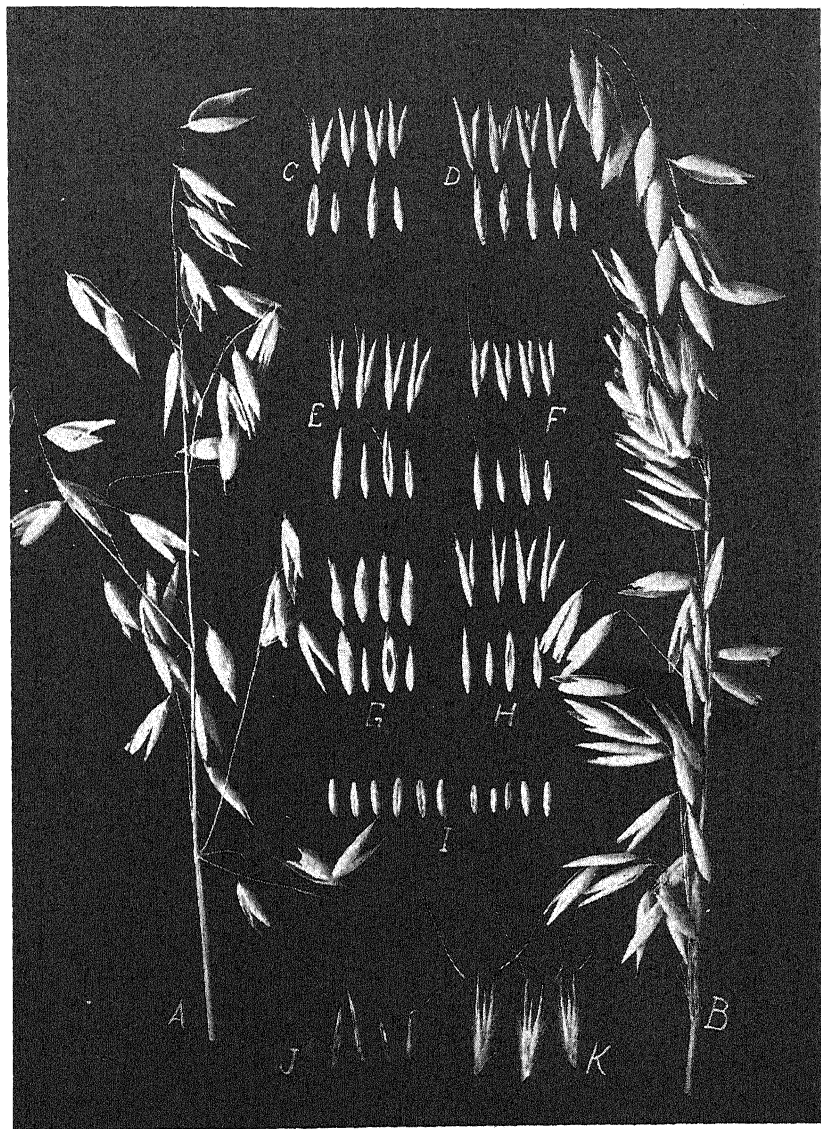


Figure 1. Panicles, spikelets, and florets of oats: (A) Equilateral (spreading or open) panicle; (B) unilateral (side or horse-mane) panicle; (C) common oats; (D) red oats; (E) long grains; (F) short grains; (G) plump grains; (H) slender grains; (I) hull-less or naked oats; (J) common wild oats (*Avena fatua* L.), and (K) wild red oats (*A. sterilis* L.).

In regard to the botany of oats, Stanton wrote as follows:

"Under average conditions, the oat plant produces from three to five hollow stems, or culms, varying from one-eighth to one-fourth inch in diameter and from 2 to 5 feet in height. The roots are small, numerous, and fibrous, and penetrate the soil to a depth of several feet. The leaves average about 10 inches in length and five-eighths of an inch in width. The panicles, or heads, are either spreading (equilateral or treelike) or one-sided (unilateral, horse-mane, or bannerlike). By far the greater number of cultivated varieties are of the type with spreading panicles. The grain is produced on small branches, in spikelets, varying in number from 20 to 150 per panicle. The number of florets or grains in each spikelet, except in the hull-less or naked oat, varies from two to three. The spikelet is loosely enclosed within the outer glumes (chaff). The kernels, except in the hull-less oat, are tightly enclosed within the lemmas or inner glumes and palea. The lemma or hull varies in color from white, yellow, gray, and red to black and may be awned or awnless. The kernel, or more properly the caryopsis, without its adhering glumes, is very slender, ranging from five to seven sixteenths of an inch in length and from one to two sixteenths of an inch in width. The kernel constitutes about 65 to 75 percent of the total weight of the whole grain."

Histology of the Oat Seed

Microscopic study of many kinds of seeds has been thorough and exhaustive. Drawings of the detailed interior and exterior structure of kernels of maize and wheat have been published in both lateral and longitudinal sections through different parts of the kernels, appearing as early as fifty years ago. One of the finest old works on detailed structure of the cereal grain seeds is the German text "*Landwirtschaftliche Samenkunde I, II*," by Harz (1885). That book, however, fails to show the structure of the oat kernel in longitudinal section through the embryo. Avery (1930) portrayed particularly the very highly magnified oat embryo. For those interested especially in the comparative structure of maize, oats, and wheat kernels Avery's article should prove extremely useful.

Reference to the extensive works of the contemporary authors Andrew L. Winton and Kate Barber Winton (1932) disclosed that their well-known book, "*Structure and Composition of Foods, Volume 1*," did not describe the oat kernel in great detail. Contact was therefore established with the Wintons and since they could recall from the literature no thorough drawing of a longitudinal section showing all important parts of the oat kernel, A. L. Winton was commissioned to make that study. Because of its typical character, its growing popularity and its practical importance, the Iogold variety was selected for the microscopic studies. Development of Iogold oats was started at Iowa State College about 1906 from a single plant of the Kherson variety. Particularly favorable characteristics of Iogold oats are the stiffness of straw, high yield, and excellent resistance to parasitic stem rust. L. C. Burnett, who generously furnished materials for this histological study, has described Iogold oats thoroughly in a bulletin of the Iowa State College Experiment Station (1928).

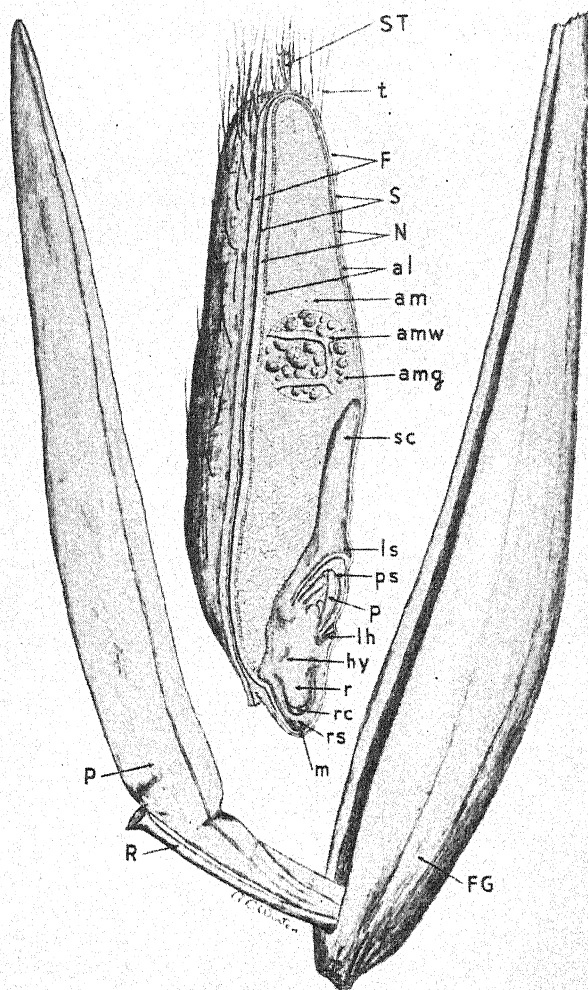


Figure 2. Oats (*Gramineae Avena sativa*). The drawing reproduced above shows a kernel of logold oats, lifted out of its enveloping chaff or hulls (flowering glumes and palea), partially sectioned longitudinally through the cleft and with a very restricted part of the endosperm further enlarged as an inset. The drawing was made by Andrew L. Winton, sometime State and Federal Chemist, co-author of "The Structure and Composition of Foods" in four volumes (John Wiley & Sons, Inc.), and other publications.

The scale of original drawing was 22 times natural size for all parts except the inset, which was 300 times natural size; as printed above, the scale is 11 times natural size for all parts except the inset, which is 150 times natural size.

Naming and lettering are done in accord with the internationally convenient policy of adhering as closely as possible to the Latin. Capital letters distinguish morphological parts such as fruit coat, seed coat, and others, while the smaller letters indicate layers of cells and their contents making up such parts.

Parts of kernel or groat: (ST) remnant of style, (t) trichomes or hairs, (F) fruit-coat or pericarp, (S) spermoderm or seed-coat, (N) hyaline layer or perisperm, (al) aleurone layer of endosperm, (am) amylaceous or starchy endosperm, (sc) scutellum or cotyledon of embryo (germ), (ls) ligule of embryo, (ps) plumule sheath, (p) plumule or leaf bud, (lh) leaf bud of hypocotyl, (hy) hypocotyl, (r) radicle or embryonic root, (rc) radicle cap, (rs) radicle sheath, (m) micropyle or opening through which the pollen entered.

Inset: Aggregates of starch grains in starch cells. (amw) starch cells; (amg) starch grains (numerous).

Hundreds of kernels and sections of kernels were observed by A. L. Winton and from them the pencil drawing shown in Figure 2 was made. Winton (1936) wrote in regard to the drawing:

"The two novel features which the picture brings out are the long scutellum and the transition from transversely elongated starch cells above to the isodiametric cells below."

The explanation, legend and lettering given in Figure 2 are also in accord with the designations by Winton.

Official Grain Standards Regarding Oats

For the purposes of the United States Grain Standards Act the United States Department of Agriculture (1936) has described oats in part as follows:

Oats shall be any grain which consists of 80 percent or more of cultivated oats. Oats may contain not more than 10 percent of wild oats.

Oats are further classified according to color as follows:

Class I, White Oats; Class II, Red Oats; Class III, Gray Oats; Class IV, Black Oats; and Class V, Mixed Oats.

It is pointed out that "White Oats" shall include yellow oats. Other details of the oat standards concern themselves with soundness, brightness, and degree of infestation of the grain.

Official and non-official definitions of oats and their milled products follow after the section on milling.

Milling of Oats

Oat-milling procedure is especially adjusted to the structure of the oat kernels and of the chaff (or hulls) which closely envelopes each individual kernel through the threshing and well into the milling at the processing plant. A mill equipped effectively to process oats could not satisfactorily mill wheat and the converse is equally true.

Oats are purchased from country or terminal grain elevators subject to inspection to assure that only oats of suitable high grade enter the mill. Soundness of kernels and freedom from heat damage, foreign odors, wild onion seed, smut, must, and molds are of vital importance. At the mill oats are placed in large elevators. Moisture content of more than 13% is likely to cause heating and discoloration damage. To guard against that, moving and aeration of grain to lower its temperature can be accomplished when necessary.

The oat mill flow-sheet, as shown in Figure 3, indicates the most important steps in the processing of oats. Sunderman (1931) has also discussed oat-milling methods. The initial step—cleaning of the oats—removes foreign materials such as metallic pieces, dust, chaff, weed

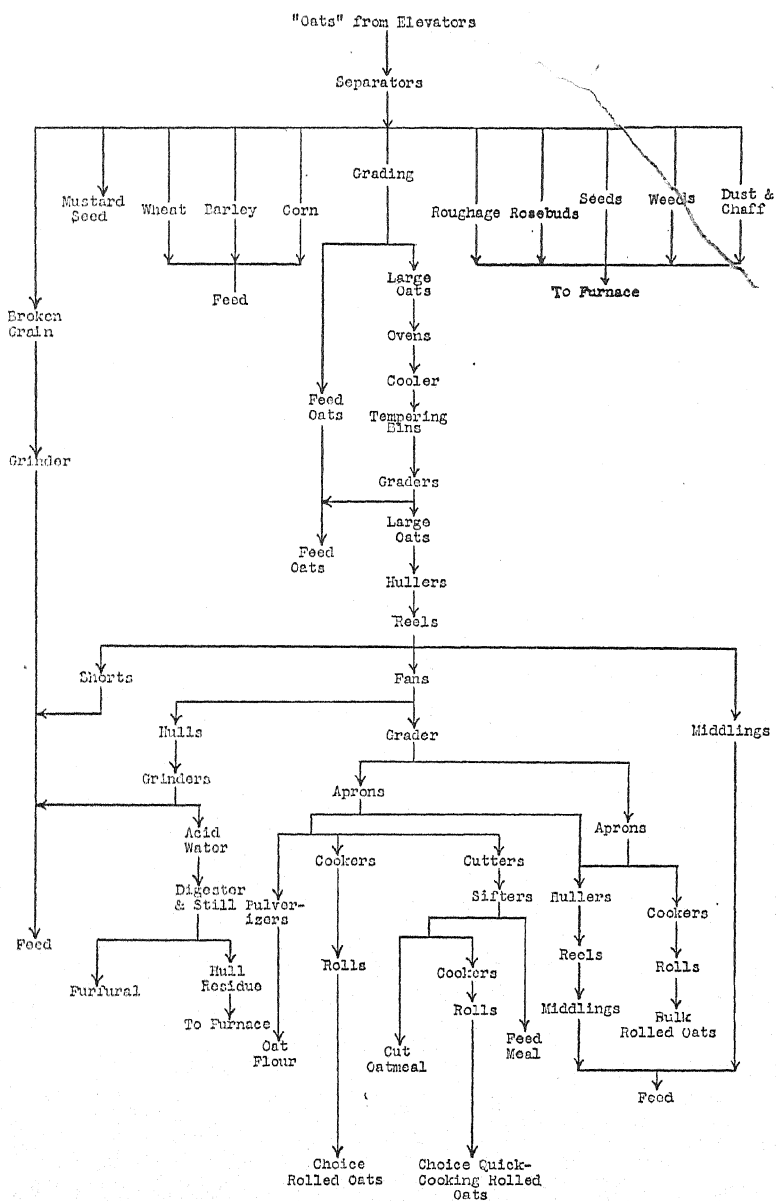


Figure_3. Oat mill flow-sheet.

seeds, wheat, corn and barley, as well as oats which are either small, light or double. Some thirty different machines, or separators, are employed to remove these foreign materials. The separators serve specific purposes and are of the following general types: air, disc, grading reel, grading separator, apron, cylindrical, centrifugal, mustard seed, and magnetic.

When the cleaning is completed the oats are conveyed to the so-called drying house where the moisture content is reduced from about 10 or 12% to about 6%. Desiccation is accomplished in a vertical stack of large open steel pans, the bottoms of which are steam jacketed. Oats fed to the center of the top pan are slowly stirred outward by a sweeping paddle which moves them toward the edge of the pan where they drop into a chute and are directed to the middle of the next lower pan. This procedure is repeated from 8 to 12 times. In the trade various names such as roasting, toasting, dry cooking, or cooking are sometimes applied to that treatment. The roasting stage is of approximately one-hour duration throughout which a temperature of substantially 180° F. is maintained in the oats. This heating makes the hulls more brittle, thus facilitating their subsequent removal, and in addition the slow roasting develops a certain desirable flavor in the groat which persists into the final product.

From the bottom of the roasting stack the oats which are still tightly enveloped individually by the hulls are taken through an air cooler. The oats are then ready for the oatmeal mill proper in which the chief steps are: separation of oat grains into grades closely identical as to diameter and length; hulling; separation of the hulls and the unhulled oats from the groats; steaming the groats preparatory to rolling; rolling the groats into flakes; and packaging.

In the precise separations of oats according to size the largest kernels usually constitute a majority and are designated "large oats," while other fractions are called "slim oats," "long slims," "No. 1 stub," "No. 2 stub," and "No. 3 stub," respectively. Each of these streams is sent separately to the hulling stones, the "large" oats going to make the choicest grade of large flakes, while the less choice or smaller oats may be converted into so-called bulk rolled oats.

One of the most fascinating operations in an oat mill is the hulling of the grains, the tearing of the hulls or glumes and paley from the kernels or groats. To remove the hulls the oats are passed between two large circular stones about three feet in diameter, mounted horizontally one immediately over the other. The upper surface of the lower stone is almost flat while the lower surface of the upper stone is slightly conical. The upper stone rotates rapidly. The lower stone is stationary. Oats under flow of gravity pass through an opening in

the center of the upper stone and are gradually forced to the outer edge of the pair of stones. The stones are precisely adjusted so that the intervening space is just slightly greater than the length of the groats in the grains to be hulled. En route between the center and outer edge of the stones the grains are turned on end. Hulls are shattered and torn from the groat. The yield of unbroken groats is determined by the skill and perfection with which the stones are "dressed" and adjusted. Formerly the hullers were actually of stone but now they are of manufactured silicon carbide composition.

From the hulling stones the mixture of loosened hulls, whole oat groats, and bits of floury pieces broken from the groat are directed through sieves, reels and purifiers which make sharp separations of the various fractions. The oat groats obtained at this point may be processed by any one of a large variety of methods each of which would give rise to a distinctive food, feed, or industrial product. While a small amount of whole-oat groats is used in that form for special feeding purposes, most of the groats are transformed to other products. Of greatest prominence among products made from oat groats are the flakes or rolled oats. Whole groats destined for the larger ("standard") flake are steamed directly with live steam at atmospheric pressure for a few minutes preceding the rollers. This softens the kernels so that pulverization is kept to a minimum when the groat is flattened as it passes between precisely adjusted heavy steel rolls.

If, in contrast, the groats are to be made into the so-called quick-cooking or smaller type flakes, it is first necessary to steel-cut the groats into pieces by means of rotary type granulators. Several distinct degrees of granulation are thus obtained which are often classified simply as A (finest), B (medium), and C (coarsest) steel-cut or "cut oats." Steel-cut or "Scotch Oats" were at one time the chief form in which oats were consumed as a human food, being displaced later by rolled oats. In the medium steel-cut oats each granule represents about one-third of a groat. That is the size usually employed for making the quick-cooking rolled oats, one granule making one flake. One entire groat is required to make one flake of the larger "standard" or slower cooking type of rolled oats. The rolling of the medium steel-cut oats is accomplished by technique identical with that used for the larger flakes except that the rolls are set closer together to make thinner flakes. Heat treatment of the two kinds of flakes is similar.

Milling percentage of rolled oats varies widely. It is dependent especially on quality of the grain. The heavier, plumper, dryer, and cleaner oats give the best milling yield. The skill of the millwrights in dressing and adjusting the hulling stones, as well as in operating numerous other machines, has great influence on the yield of rolled oats.

With medium good quality No. 2 White Oats as they are usually received at the mill, about 13.5 bushels or 432 pounds are required to produce a barrel of good quality rolled oats. A "barrel" of rolled oats or oat groats is 180 pounds. In contrast, a "barrel" of ground oatmeal or steel-cut oats is 196 pounds.

Definitions of Oats and Certain of Their Milled Products

The only official definitions of oat products are those by the Association of American Feed Control Officials, Inc. (1937). Their definitions regarding oat products are reproduced in full as follows:

- "*Oat Hulls* is the product consisting of the outer covering of the oat.
- "*Oat Middlings* is the product consisting of the floury portions of the oat groat obtained in the milling of rolled oats.
- "*Oat Shorts* is the product consisting of the covering of the oat grain lying immediately inside the hull, being a fuzzy material carrying with it considerable portions of the fine floury part of the groat obtained in the milling of rolled oats.
- "*Oat Chop, Ground Oats, Pulverized Oats, Crushed Oats, or Crimped Oats* consists of the entire product made by chopping, cutting, grinding, crushing, or crimping whole oats.
- "*Oat Groats* are the kernels produced from cleaned and dried oats in the process of manufacturing oat meal.
- "*Hulled Oats or Undried Oat Groats* are the kernels produced from the undried grain in the process of hulling oats.
- "*Oat Meal or Ground Oat Groats* is the product produced by cutting, cracking or grinding oat groats.
- "*Rolled Oat Groats or Rolled Oats* is the product obtained in the process of rolling oat groats.
- "*Clipped Oat By-Products* is the by-product obtained in the manufacture of clipped oats. It may contain the light chaffy material broken from the end of the hulls, empty hulls, light immature oats and dust. It must not contain an excessive amount of oat hulls.
- "*Oat Mill Feed* (Oat Hulls, Oat Shorts and Oat Middlings) is the entire by-product produced in the manufacture of oat groats and consists of oat hulls, oat shorts and oat middlings. If used in a mixed feed, it shall be called Oat Mill Feed (Oat Hulls, Oat Shorts and Oat Middlings)."

In the absence of official definitions covering still other materials of importance, it seems advisable to describe them non-officially as follows:

Oats—often refers to cereal grass plants of the *Gramineae Avena sativa* classification. The word may also mean, according to equally common usage, the grains of oats with their hulls still intact. It is in this form that the grains leave the thresher or "combine" harvester and continue through several of the early stages of processing at the oat mill.

Ground Oats—the grains including hulls and groats in their natural proportions, ground to a mealy character generally in an attrition mill.

Ground Oat Meal—oat groats ground to a mealy consistency in an attrition mill.

Steel-cut Oats, Cut Oats, or Scotch Oats—oat groats cut by special steel rolls into granular pieces varying in size according to arbitrary

trade specifications dependent on size of granules. Three sizes are commonly designated, namely: A—finest, B—medium, and C—coarsest.

Rolled Oats—oat kernels (groats), or steel-cut (cut) oats including endosperm, bran and germ which have been pressed between two rolls in such a manner as to produce flattened discs. There are substantially two well-known types of rolled oats. "Regular" or "standard" rolled oats are the flakes produced by rolling whole oat groats, in which case one whole groat produces one large flake. "Quick-cooking" rolled oats are the smaller, thinner flakes produced by rolling steel-cut oats. Usually the medium-sized cut oats are used to make quick-cooking flakes, which are both thinner and much smaller in diameter than are "standard" rolled oats.

Oat Flour—finely ground and bolted flour made from oat groats. A variety of oat flours is produced. Depending on the use for which they are designed, the range of flours varies chiefly in texture, color, and fiber content.

Oatmeal—a term which has a wide variety of meanings. Literally, it means the meal of oats. It is also used less specifically to designate steel-cut or rolled oats. The term may also be used to indicate the porridge made from any of these three general forms of oat foods. However wide and non-specific the term may have become, the circumstances of its use generally give automatic intimation as to the precise product concerned.

Composition of Oats and Certain Oat Products

Figures typical of the chemical composition of oat grains, oat kernels (groats), and of rolled oats are reproduced in Table II, from Sherman's book on *Food Products* (1933).

TABLE II
ANALYSES OF OATS AND OAT PRODUCTS

	Oat grains, kernels with hulls	Oat kernels, without hulls	Rolled oats
	%	%	%
Moisture	10.06	6.93	7.7
Protein	12.15	14.31	16.7
Fat	4.33	8.14	7.3
Fiber	12.07	1.38	1.3
Carbohydrates, other than fiber	57.93	67.09	66.2
Ash	3.46	2.15	2.1

Further data, concerning particularly the approximate mineral content of rolled oats as reported by Miner (1926, 1933), are indicated in Table III.

TABLE III
APPROXIMATE MINERAL CONTENT OF ROLLED OATS

Calcium, expressed as CaO	0.083%	Chlorine, expressed as Cl	0.090%
Phosphorus, expressed as P ₂ O ₅	0.920%	Magnesium, expressed as MgO	0.260%
Iron, expressed as Fe ₂ O ₃	0.0043%	Potassium, expressed as K ₂ O	0.200%
Copper, expressed as CuO	0.000423%	Silicon, expressed as SiO ₂	0.049%
Manganese, expressed as Mn ₃ O ₄	0.0098%	Sodium, expressed as Na ₂ O	0.140%
Aluminum, expressed as Al ₂ O ₃	0.0123%	Sulphur, expressed as S	0.225%
Bromine, expressed as Br	0.00039%		

Oat Foods

According to citations by Thornton (1933) the entire supply of oat foods for human consumption in the United States up to about the year 1850 was imported from Scotland and Canada. Oatmeal then was sold in drug stores as a remedial agent. Starting about the middle of the nineteenth century the milling of oat foods was commenced in Ravenna, Ohio. Since that day the increase of oat foods manufacture has grown with remarkable speed.

For a period of substantially seventy or eighty years the "bowl of oatmeal" has been used with traditional regularity to designate a serving portion of America's most widely consumed porridge. In some sections of the country the words "porridge" and "oatmeal" are considered synonymous and are used interchangeably. The fact that oatmeal has been marketed in four different physical forms makes little difference to many consumers who have preferred to use the word oatmeal in its broad sense. In the earliest years of American production of oat foods the groats were ground into a meal; at a later period steel-cut oats rose to greater popularity. Steel-cut oats were followed by the large flakes and latterly they in turn have been partially supplanted by the smaller or quick-cooking type of flakes. Today the chief food products of an oat mill are the two kinds of flakes or rolled oats, and by far the larger proportion of these are packed in fiber packages in contrast with the barreled, or sacked, oatmeal of earlier periods. The smaller flakes continue to win the favor of many consumers because they are more quickly cooked. The starch of the smaller flakes is more rapidly swollen and ruptured because the thinness permits more ready penetration by the hot water and steam.

The composition of oat foods of all shapes or forms is substantially similar. Therefore, one set of figures serves satisfactorily for oat groats, steel-cut oats, ground oats and most oat flours. Tables II and III show these data for oats and oat products. Table IV indicates composition of oats and their chief milled products in comparison with corresponding data for five other cereal grains.

TABLE IV
APPROXIMATE COMPOSITION OF CEREAL GRAINS AND OF CERTAIN
CEREAL FOODS

Grain or product *	Moisture	Protein	Fat	Crude fiber	Carbohydrate ¹	Ash	Calories	
							Per 100 grams	Per ounce
<i>Barley (Gramineae Hordeum sativum—family, genus, species)</i>	%	%	%	%	%	%		
Entire grains with hulls	11.9	10.5	2.2	3.8	72.8	2.6	350	100
Pearled barley	10.8	8.5	1.1	0.5	77.8	1.3	355	101
<i>Corn (Gramineae Zea mays)</i>								
Entire grains or kernels	10.7	10.0	4.3	1.7	71.8	1.5	366	104
Degerminated corn meal	11.0	7.8	1.3	0.8	78.5	0.6	357	101
<i>Oats (Gramineae Avena sativa)</i>								
Entire grains with hulls	10.1	12.2	4.3	12.1	57.8	3.5	319	90
Entire kernels without hulls (groats), oatmeal (ground), or rolled oats	8.7	16.0	5.6	1.2	66.7	1.9	380	108
<i>Rice (Gramineae Oryza sativa)</i>								
Entire grains with hulls, "Rough rice"	11.7	8.1	1.8	8.9	64.3	5.2	305	86
Brown rice (entire kernels, without hulls)	12.1	9.1	2.0	1.1	74.6	1.1	353	101
Polished rice	11.5	7.0	0.6	0.8	79.3	0.8	352	100
<i>Rye (Gramineae Secale cereale)</i>								
Entire kernels without hulls	9.4	11.8	1.8	1.8	73.2	2.0	356	101
Rye flour	11.1	7.9	1.1	0.4	78.7	0.8	356	101
<i>Wheat (Gramineae Triticum sativum)</i>								
Entire kernels without hulls	10.3	12.4	2.2	2.2	71.0	1.9	353	101
Patent flour (white)	11.0	11.0	1.3	0.2	76.1	0.4	360	102

¹ Calculated by difference—crude fiber excluded.

As a source of vitamins oats have a trace of vitamin A, they are a very good source of vitamin B₁, they are devoid of vitamins C and D, their content of riboflavin and other parts of the vitamin G (B₂) complex is uncertain, and they are considered to contain appreciable vitamin E. Vitamin B₁ assays have been made in the laboratory of one of the authors on successive stages of oats from the grain elevator to rolled oats porridge. It was found that roasting for flavor development did not impair the B₁ content, that there was a slight loss at the flaking stage, and a further slight loss on cooking to a porridge. The figures in terms of International units of vitamin B₁ per ounce (on the air-dry basis) are respectively: raw oats 62, "toasted" oats 62, oat groats 62, rolled oats 54, and rolled oats porridge or oatmeal 47. For comparison it can be indicated that the vitamin B₁ potency of raw, unheated, unmilled whole wheat has been found in simultaneous tests

0

OATS AND OAT PRODUCTS

Vol. 15

o assay approximately 40 International units per ounce. Thus it is evident that rolled oats porridge is a very good source of vitamin B₁.

The greatest consumption of oat foods occurs in the so-called "breakfast food" group. As used for that purpose for children and adults alike the cooking methods employed vary widely from family to family. In general, one ounce of rolled oats produces about four ounces of porridge. That amount constitutes a typical serving portion which is usually eaten with added milk or cream either with or without a bit of sugar. Rolled oats are also used in numerous other culinary capacities, as for example cookies, breads, and puddings.

National production figures for oat breakfast foods provide the closest indices available concerning the annual consumption of these foods. Exportation and importation of "breakfast foods" from this country are so small as to be of little significance. Latest Federal statistics on production compiled by the United States Department of Commerce (1937) are indicated in Table V. These figures concern the production year 1935.

TABLE V

CEREAL "BREAKFAST FOOD" PRODUCTION IN THE UNITED STATES DURING 1935

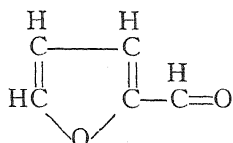
Breakfast foods:	
Total pounds.....	1,151,546,585
Total value.....	\$99,344,148
Made from wheat:	
Pounds.....	396,067,131
Value.....	\$45,537,950
Made from oats:	
Pounds.....	528,934,528
Value.....	\$26,386,380
Made from corn:	
Pounds.....	184,348,608
Value.....	\$18,792,603
Made from other grains and from mixed grains:	
Pounds.....	42,196,318
Value.....	\$8,627,215

The data show that oat foods, substantially all of which are rolled oats, constituted more than 46% of all breakfast foods produced. It is interesting, especially in view of the more recent findings and opinions in regard to nutrition and dietetics, that all of the oat foods are whole-grain foods containing the bran and germ as well as the endosperm portions of the kernels. Corn breakfast foods are almost entirely "refined" in the sense that the bran and germ have been removed. Of the wheat products it may be estimated that approximately one-third of the wheat breakfast foods are whole-grain foods. On the basis of these figures one can then indicate that the American public consumes its whole-grain foods in approximately the following quantities per

Oat breakfast foods.....	528,934,528	pounds
Wheat breakfast foods.....	132,022,377 ⁴	pounds
Graham and whole-wheat flour.....	307,431,684 ⁵	pounds

Other uses beyond the fields of human food and livestock feed have been found for various oat products. Among these are the chemical compound furfural and its derivatives which are made from oat hulls, oat flour as an agent to retard rancidity development in foods, and oat flour as either an ingredient in soaps or as detergent agent used in lieu of soaps. Since circumstances do not permit a detailed review of each of these uses here, it will perhaps suffice to describe those uses briefly and provide references to more exhaustive publications covering the respective phases of oats.

Furfural is a liquid aldehyde, light amber in color, having an odor which is not unpleasant. Furfural is made by destructive distillation of oat hulls in the presence of acid and steam under regulated conditions. In that way the pentosans are cleaved first to pentoses and then dehydrated to furfural. The structural formula of furfural follows:



The anatomical differences between grains of oats and wheat, particularly the fact that the oat hulls are not removed in threshing, is basically responsible for the furfural industry's close connection with oats. The corresponding hulls of wheat, in contrast, readily yield to separation from the kernels when threshed. Because of its aldehyde structure which condenses successfully with phenols, ketones, or amines to form resins, many of the widely used plastics are now made in part from furfural. Its solvent properties have commended it to use in paint removers, lacquer solvents, as selective solvents for purifying petroleum lubricating oils and for numerous other purposes. A review of furfural and other furans together with a considerable bibliography was published by Peters (1936).

Oat meal (or flour) soaps and bath "packs" have been used for years, both for ordinary cleansing and beautifying purposes as well as for treatment of dermatitis and eczema. As a constituent of certain soaps, the oat meal adds pleasant and effective detergent qualities. Latterly there has been produced a perfumed oat flour which is used both for therapeutic and for more widespread cosmetic applications either in bath water or directly to the skin as a powder or cream.

⁴ One-third of wheat volume figure shown in Table V.

⁵ Taken from the 1935 production figure given on page 5 of the previously cited Bulletin by the United States Department of Commerce.

Oat flour has the effect of retarding rancidity in certain foods, particularly those high in fat content, and is gaining increased usage therefore particularly in the field of human food and animal feed where higher potency chemical antioxidants are objectionable because of toxicity. Recent progress in this further expansion of uses for oat flour is summarized in a review by Peters and Musher (1937).

Summary

Oats, *Gramineae Avena sativa*, is an important human food and livestock feed crop of this country. In terms of tonnage produced in 1935 it ranks immediately ahead of wheat and is second only to corn in the cereal group. This article includes original publication of a drawing by A. L. Winton showing detailed microscopic structure of the seed (or kernel) of Iogold oats in partial longitudinal section. The method of milling oats, descriptions and definitions of related products, composition and uses of the oat foods, feed materials, and industrial products are discussed.

Literature Cited

- Association of American Feed Control Officials, Inc.
 1937 Official Publication, 20-21.
- Avery, George S., Jr.
 • 1930 Comparative anatomy and morphology of embryos of seedlings of maize, oats and wheat. *Botan. Gaz.*, lxxxix, 1: 1-39.
- Burnett, L. C.
 1928 Iogold Oats. *Agr. Expt. Station, Iowa State College, Bull.* 247.
- Denaiffe and Sirodot.
 1902 L'Avoine. J. B. Baillière and Sons, Paris.
- Harz, C. O.
 1885 *Landwirtschaftliche Samenkunde I, II.* P. Parey, Berlin.
- Miner, C. S.
 1926 and 1933 Private communications.
- Peters, Fredus N., Jr.
 • 1936 The furans, fifteen years of progress. *Ind. Eng. Chem.* 28: 755-9.
 — and Musher, Sidney
 • 1937 Oat flour as an antioxidant. *Ind. Eng. Chem.* 29: 146-151.
- Sherman, H. C.
 1933 *Food Products*, Third Edition. Macmillan.
- Stanton, T. R.
 1936 Superior germ plasm in oats. *U. S. Dept. Agr. Yearbook of Agriculture*, p. 347-414.
- Sunderman, R.
 • 1931 Selection and grading requisite to oatmeal and production. *Food Industries* 3: 372-5.
- Thornton, H. J.
 1933 *The History of The Quaker Oats Company.* Univ. of Chicago Press.
- United States Department of Agriculture
 1936 *Handbook of Official Grain Standards of the United States.* U. S. G. S. A. —Form No. 90.
 1936a *Yearbook of Agriculture, Statistical Supplement.*
- United States Department of Commerce
 1937 *The census of manufacturers, 1935. Flour and other grain-mill products, cereal preparations, prepared feeds, etc.*
- Winton, Andrew L., and Winton, Kate Barber
 1932 *The Structure and Composition of Foods. Vol. I. Cereals, Starch, Oil Seeds, Nuts, Oils, Forage Plants.* John Wiley & Sons.
 1936 Private communication.

THE NATURE OF THE INCREASE IN AMYLASE ACTIVITY OF GERMINATING BARLEY¹

CLAUDE H. HILLS and C. H. BAILEY

Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

(Read at the Annual Meeting, May 1937)

The increase in amylase activity which occurs upon the germination of barley and other cereal grains was attributed by early investigators to the actual synthesis of enzymes, as indicated by Lüers (1936) in his review of the earlier investigations. The results of more recent researches show that this concept is probably erroneous.

Ugrumow (1935) found that both α - and β -amylases were present in the earlier stages of development of wheat kernels. The α -amylase activity disappeared entirely as the grain passed through the "milk-ripe" stage and did not appear again until the third day of germination. The β -amylase activity of the maturing kernels decreased slightly until it reached a minimum at full maturity. The increase in β -amylase activity began at the end of the steeping period in the malting operation and continued until an increase of approximately 100% was observed by the second week of germination.

Chrzaszcz and Janicki (1936) have shown that this sequence of changes in amylase activity during ripening and subsequent germination is characteristic also for barley and other cereals. It was observed that the dextrinizing power of ripening barley disappeared completely during the "milk" stage and did not appear again until the fourth or fifth day of germination.

There have been several attempts in recent years to explain the increase in diastatic activity observed on germination of barley. Waldschmidt-Leitz and Purr (1931) presented evidence to show that this increase may be caused by the formation on germination of barley of an amylase activator which they have designated as "amylokinase." The activator was separated from the amylases of green malt extracts by adsorbing the amylases from solution with Tonerde C γ at pH 5.0. When this concentrated amylase-free activator solution was added to an extract of ungerminated barley, they observed a 200% increase in maltose production.

¹ Paper No. 1549, Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Claude H. Hills to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1937.

The "amylokinase" was non-diffusable through animal membranes and was not entirely destroyed by heating to 100° C. for 30 minutes. Thus it may be of the nature of a protein degradation product. These authors concluded that the "amylokinase" activated both α - and β -malt amylases, but had no effect on pancreatic amylase.

Lüers and Rümmler (1935) were unable to confirm Waldschmidt-Leitz and Purr's results. A similar inability to confirm the "amylokinase" hypothesis was reported by Weidenhagen (1933) who stated further that the activator had no action on pure amylases but protected against inhibitory substances in crude extracts.

Chrzaszcz and Janicki (1933b) explained the changes in amylase activity on germination of barley by postulating the presence of an amylase inhibitor, "sisto-amylase." The increase in amylase activity on germination was attributed to the ability of the protein degradation products to counteract the effect of the "sisto-amylase" and thus restore the full activity of the amylases. The action of this inhibitor could be counteracted by certain "eleuto-substances" such as peptone or NaCl. Chrzaszcz and Janicki (1936) attributed the decrease in amylase activity during the ripening of barley to an accumulation of "sisto-amylase."

The concentration of "sisto-amylase" in barley or malt was determined by measuring the increase in amylase activity upon the addition of peptone to the raw extracts. The fact that "sisto-amylase" occurred in small amounts in ungerminated barley and increased rapidly on germination seems to be inconsistent with the conclusion that the decrease in amylase activity on ripening of cereals was due to an accumulation of the inhibitor.

The studies of Oparin, Manskaja and Magaram (1933) led to a different interpretation of the effect of peptone on barley malt extracts. They observed that egg albumin increased the thermo-lability of amylase in solution. If the added egg albumin was denatured by physical or chemical means it adsorbed part of the soluble amylase on the insoluble protein precipitate. Peptone partially protected malt amylases against this type of inactivation.

Oparin, Manskaja, and Glasunow (1934) concluded that a tannic acid precipitate of egg albumin, when added to a barley malt extract, behaved like the "sisto-amylase" described by Chrzaszcz and Janicki. They stated the belief that peptone peptized the amylase adsorbed on insoluble protein.

Ford and Guthrie (1908) first demonstrated that the digestion of barley extracts with papain increased amylase activity. More recently Myrbäck and Myrbäck (1933) employed this technique to de-

termine the "free" and "bound" amylase content of barley. By digesting a barley meal suspension with papain they observed a 200% to 300% increase in amylase activity. Thus they explained the increase in β -amylase activity on germination of barley as due to the proteolytic release of insoluble amylase bound to protein.

Experimental

The existence and possible nature of anylokinase was investigated by repeating some of the original experiments of Waldschmidt-Leitz and Purr (1931, p. 124). They removed the amylases from a green malt by adsorbing with Tonerde Cy. When 8 c.c. of this amylase-free activator solution were added to .5 c.c. of barley extract they observed that the maltose production increased from 37.7 mg. to 120 mg.

The green malt used by us was prepared by steeping a 300 g. portion of barley in cold running water at 12.5° C. for 52 hours and then germinating in a small laboratory malting cabinet for 6 days at 16° C. The malted barley was frozen, ground through a food-chopper, and stored at -20° C.

Green malt extract: A green malt extract was prepared by extracting 2.5 g. (dry basis) of frozen malt with 50 c.c. of water, 3 hours at 21° C. The mixture was centrifuged and the extract decanted.

Amylokinase solution: To 25 c.c. of extract prepared from frozen green malt there were added 5 c.c. of 1.0 N acetate buffer (pH = 5.0). This extract was treated with four successive additions of 250 mg. (14.5 c.c.), 125 mg., 50 mg., and 50 mg. of Tonerde Cy. After each addition of Tonerde Cy the mixture was allowed to stand for 5 minutes, and then centrifuged at half speed for exactly 5 minutes and the supernatant liquid decanted. After the last decantation the volume was made up to 50 c.c.

Barley extract: Ungerminated barley was ground through two series of corrugated rolls. To 2.5 g. of ground barley 50 c.c. of water were added, and the mixture extracted 3 hours at 21° C. and centrifuged. The extract was diluted 1:10 with water.

Starch substrate: A solution containing 25 c.c. of 2% soluble starch + 10 c.c. of 0.1 N citrate buffer (pH = 5.1) + enzyme additions + water to make 46 c.c. final volume, was digested 30 minutes at 37° C. Then 2 c.c. of 10% H_2SO_4 and 2 c.c. of 12% Na_2WO_4 were added. Maltose was determined in a 5 c.c. aliquot by Blish and Sandstedt's (1933) modification of the Hagedorn-Jensen method.

These results show no increase in maltose production due to the activator solution. The activator solution still contained a trace of

TABLE I
EXPERIMENTS DESIGNED TO DETECT AMYLOKINASE IN GREEN MALT EXTRACTS

Number	Enzyme addition	Experiment I ¹	Experiment II
		Mg. maltose per 50 c.c.	
1	2 c.c. barley extract	92.6	93.0
2	8 c.c. activator solution	6.0	13.6
3	Sum of No. 1 + No. 2	98.6	106.6
4	2 c.c. barley extract + 8 c.c. activator solution	93.0	95.4
5	Net increase due to activator (No. 4—No. 3)	-5.6	-11.2
6	% hydrolysis of starch substrate in No. 4	18.6%	19.1%
7	1 c.c. green malt extract diluted 1 : 10	100.0	106.0
8	Calculated % removal of amylases from activator solution	99.85%	99.70%

¹ Tonerde C₇ used in Experiment I was obtained from Waldschmidt-Leitz.

amylase activity although 99.7% to 99.85% of the amylases has been removed.

In a further effort to detect amylokinase in green malt extracts the above experiments were repeated with two minor changes in the procedure: fresh unfrozen green malt was used instead of frozen malt,

TABLE II
FURTHER EXPERIMENTS TO DETECT AMYLOKINASE IN GREEN MALT EXTRACTS

Number	Enzyme addition	Number of experiment				
		IV	V	VI ¹	VII ¹	VIII ¹
		Mg. maltose per 50 c.c.				
1	2 c.c. barley extract	85	91	90	93	88
2	8 c.c. activator solution	7	26	36	82	154
3	Sum of No. 1 + No. 2	92	117	126	175	242
4	2 c.c. barley extract + 8 c.c. activator solution	83	112	118	151	203
5	Net increase due to activator (No. 4—No. 3)	-9	-5	-8	-24	-39
6	% hydrolysis of starch in No. 4	16.6%	22.4%	23.6%	30.2%	40.6%
7	2 c.c. green malt extract diluted 1 : 10	97	111	128	123	116
8	% removal of amylases from activator solution	99.65%	98.83%	94.40%	86.7%	73.5%

¹ Two cubic centimeters of activator solution used in No. 2 and No. 4 instead of 8 c.c.

and smaller quantities of Tonerde C_γ were used since there was a possibility that the amylokinase had been adsorbed from solution also.

Accordingly, the experiments were repeated using fresh unfrozen green malt and a series of decreasing amounts of Tonerde C_γ, with the results shown in Table II.

The activator solution for each experiment was prepared by extracting the amylases from an extract of fresh unfrozen green malt by four successive additions of Tonerde C_γ.

Experiment IV.....	200 mg. (20 c.c.), 100 mg., 40 mg., and 20 mg.
Experiment V.....	50 mg., 25 mg., 10 mg., and 5 mg.
Experiment VI.....	40 mg., 20 mg., 8 mg., and 4 mg.
Experiment VII.....	30 mg., 15 mg., 6 mg., and 3 mg.
Experiment VIII.....	20 mg., 10 mg., and 4 mg.

No increase in maltose production was observed due to the effect of the activator solution. The extent of extraction of amylases from solution ranged from nearly 100% to as low as 73.5%. These results agree with those of Lüers and Rümmler (1935) and of Weidenhagen (1933) who were unable to confirm Waldschmidt-Leitz and Purr's results.

Effect of Papain and Peptone on α - and β -Amylase Activity

Myrbäck and Myrbäck (1933) have pointed out that part of the amylases of ungerminated barley are water-soluble and a considerable part of the active amylase is insoluble in water. They employed digestion with .5% papain for 24 hours to determine the "total" amylase activity of barley. The portion soluble in water was designated "free" amylase and the insoluble portion was called "bound" amylase.

Chrzaszcz and Janicki (1933) contend that the effect of papain in increasing amylase activity is not due to proteolytic release of bound amylase. Instead it is attributed to the ability of the protein degradation products to counteract the inhibitory effect of "sisto-amylase" present in such extracts. Accordingly, they employed extraction with 1% peptone to determine total amylase activity.

These two hypotheses have been tested by the experiments outlined in Tables III and IV. The α - and β -amylase activities were determined in the following manner:

β -Amylase activity: β -amylase activity was determined on barley and green malt extracts prepared by extracting 2.5 g. (dry basis) of barley or malt with 50 c.c. of water for 3 hours at 21° C. The extract obtained on centrifuging was diluted 1:10 with water. Then 2 c.c. of the diluted extract were added to 35 c.c. of 2% soluble starch containing 0.1 N acetate buffer (pH=4.65). Additional 0.1 N acetate

buffer (pH = 4.65) was added to make a total volume of 46 c.c. and the mixture digested 1 hour at 30° C. At the end of 1 hour, 2 c.c. of 10% H_2SO_4 and 2 c.c. of 12% Na_2WO_4 were added and maltose determined on a 5 c.c. aliquot by the Blish-Sandstedt (1933) modification of the Hagedorn-Jensen method.

α -amylase activity: The modification of Wohlgemuth's (1908) method was to add graduated quantities of the enzyme extracts to 1 c.c. of 2% soluble starch solution buffered at pH 4.65 contained in test tubes. After addition of the enzyme extract, additional water was added to make a total volume of 6 c.c., the tubes were shaken, and the mixture digested 1 hour at 40° C. The tubes were then placed in an ice bath and 10 c.c. of ice-cold water and 1 drop of 0.1 N iodine solution were added. The end-point was taken as a deep violet color. The Wohlgemuth value, or α -amylase activity, expresses the number of cubic centimeters of 2% soluble starch dextrinized by 1 c.c. of the original enzyme solution in 1 hour at 40° C.

The effect of papain and peptone on the extraction of β -amylase from ungerminated barley is shown in Table III.

TABLE III
EXTRACTION OF TOTAL AMYLASE FROM BARLEY MEAL BY USE
OF PAPAIN AND PEPTONE

Number	Preparation of enzyme solution	Mg. maltose per 50 c.c. of digest
1	Extracted 3 hours with water	129
2	Residue from No. 1 washed with 50 c.c. of water	18
3	Residue from No. 2 digested 24 hours with .5% papain	89
Total		236
4	Digested 24 hours with .5% papain (total amylase)	248
5	Extracted 3 hours with 1% peptone	128
6	20 c.c. of solution No. 1 plus 50 mg. peptone.....	129

Peptone did not increase the amylase activity of ungerminated barley when added to the extraction mixture (No. 5) nor when added to the clear extract (No. 6). These results are contrary to the observations of Chrzaszcz and Janicki (1933a, 1933b) who found that peptone increased β -amylase activity approximately 40-100%.

The results recorded in Table III confirm the findings of Myrbäck and Myrbäck (1936) who observed that papain digestion increased the amylase activity of ungerminated barley extracts approximately 100%, but peptone was without effect on β -amylase of barley.

It is apparent that total amylase thus determined (No. 4) is equivalent to the sum of the free amylase (No. 1) and the residual amylase (No. 2 and No. 3).

Table IV extends the investigation to both α - and β -amylases of green malt.

TABLE IV
EFFECT OF PAPAIN AND PEPTONE ON AMYLASE ACTIVITY OF GREEN MALT EXTRACTS

Number	Preparation of enzyme solution	β -amylase	α -amylase
		Mg. maltose per 50 c.c.	Wohlgemuth values
1	Green malt extracted 3 hours with water	218	91
2	Residue from No. 1 washed with 50 c.c. water	23	6
3	Residue from No. 2 digested 24 hours with .5% papain	92	4
Total		333	101
4	Green malt digested 24 hours with .5% papain (total amylase)	320	125
5	50 c.c. of solution No. 1 + 100 mg. peptone	218	113
Total α -amylase (No. 5 + No. 2 + No. 3)			123
6	Green malt extracted 3 hours with 1% peptone	218	90

The increase in β -amylase activity due to papain extraction can be explained as the release of bound amylase from the residue of a water extraction. Peptone had no effect on the β -amylase activity of green malt (No. 5 and No. 6).

However, peptone had a noticeable activating effect on α -amylase. When peptone was added to a water extract of green malt, the α -amylase activity increased from 91 to 113, or about 24%. Less than 10% of the total α -amylase was released from the insoluble residue by papain (No. 3), which is quite in contrast to the large amount of residual β -amylase. Furthermore, total α -amylase is greater than the sum of the free and bound α -amylase. Much of the increase is due to the increase in activity of the soluble α -amylase.

To further test Chrzaszcz and Janicki's hypothesis concerning "sisto-amylase," peptone was added to a solution of purified α -amylase. The α -amylase preparation was secured from Olof Stamberg of the University of Minnesota, and was a very pure product as shown by its low saccharogenic activity.

A solution containing 1 mg. of α -amylase per cubic centimeter was capable of dextrinizing 14.1 c.c. of a 2% soluble starch solution in one hour at 40° C. However, when .5% peptone was added to the α -amylase solution its dextrinizing activity increased from 14.1 to 18.2 c.c. of 2% soluble starch dextrinized per hour. The peptone alone exhibited no dextrinogenic activity.

These results show that peptone increased the activity of solutions of purified α -amylase 29.1%, which is approximately the same increase as found for green malt extracts (24%). This increase may be explained in one of two ways: First, *sisto*-amylase, which is counteracted by peptone, is present in both green malt extracts and solutions of purified α -amylase, and to the same extent; or secondly, peptone may have an activating effect on α -amylase and thus affect green malt extracts and purified α -amylase to the same extent. This latter explanation seems to be the more plausible.

Oparin, Manskaja, and Magaram (1933) maintained that peptone increased amylase activity by its ability to peptize amylase adsorbed on insoluble protein particles. The effect of peptone on α -amylase may be explained by the observation that α -amylase is less soluble in water than β -amylase. Thus peptone may serve to increase the amount of α -amylase in solution.

Summary

An investigation of the existence and possible nature of "amylokinase" failed to confirm the presence of "amylokinase" in green malt extracts of barley. Even when fresh unfrozen green malt was employed and the quantities of Tonerde C γ adsorbent were varied over a considerable range, it was not possible to detect any amylase activator of the nature of "amylokinase."

Papain digestion increased the amylase activity of ungerminated barley approximately 100%. This increase in β -amylase activity was due to proteolytic release of β -amylase associated with water-insoluble material. It is concluded that papain digestion is a valid means of determining the total β -amylase activity of barley or barley malt.

Peptone did not affect the β -amylase activity of barley or malt. However, peptone increased the α -amylase activity of a green malt extract and of a solution of purified α -amylase 24% and 29% respectively.

The increase in α -amylase activity of green malt by papain extraction was not due to the release of bound amylase as in the instance of β -amylase, but was due largely to the increase in activity of α -amylase present in solution. The action of peptone in increasing the α -amylase activity of malt extracts is not clearly understood.

Literature Cited

- Blish, M. J., and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* **10**: 189-202.
- Chrzaszcz, T., and Janicki, J.
1933 Eleutoamylase, eine Antisistoamylase-substanz. *Biochem. Z.* **263**: 250-261.
1933a Sistoamylase in Malzen verschiedener Gedreidearten und ihr wirklicher Amylasegehalt. *Biochem. Z.* **264**: 192-208.
1933b Die wirkliche und scheinbare Amylasemenge in verschiedenen Getreidearten vor und während der Keimung. *Biochem. Z.* **265**: 260-281.
1936 Amylase during the growth and ripening of grains. *Biochem. J.* **30**: 1298-1302.
- Ford, J. S., and Guthrie, J. M.
1908 Contributions to the biochemistry of barley. Part I. The amylase of resting barley. *J. Inst. Brew.* **14**: 61. Abstr. in *Chem. Abs.* **2**: 1593 (1908).
- Lüers, H.
1936 Changes in enzymes during malting. *Cereal Chem.* **13**: 153-171.
— and Rummler, W.
1935 Development of amylase during germination of barley. *Wochschr. Brau.* **52**: 9-12. Abstr. in *Chem. Abs.* **29**: 4891 (1935).
- Myrbäck, K., and Myrbäck, S.
1933 Über Gersten- und Malzamylase. *Biochem. Z.* **258**: 158-171.
1936 Über freie und gebundene Amylase in der Gerste. *Biochem. Z.* **285**: 282-289.
- Oparin, A., Manskaja, S., and Magaram, M.
1933 Über den Einfluss der Koagulation von begleitenden auf die Aktivität der Amylase. *Biochem. Z.* **265**: 21-28.
— Manskaja, S., and Glasunow, I.
1934 Die Inaktivierung der Amylase durch adsorption an Eiweissniederschlägen. *Biochem. Z.* **272**: 317-323.
- Ugrumow, P. S.
1935 α - und β -Amylase in reifenden Weizensamen. *Biochem. Z.* **282**: 74-78.
- Waldschmidt-Leitz, E., and Purr, A.
1931 Über Amylokinase, einen natürlichen Aktivator des Stärkeabbaus in keimender Gerste. *Z. physiol. Chem.* **203**: 117-131.
- Weidenhagen, R.
1933 Die Reinigung erfolgte durch Ausfällen mit Ammonium und Behandeln des Niederschlages mit Aceton. *Z. ver. deutsch. Zucker-Ind.* **83**: 505-515. Abstr. in *Ergebnisse der Enzymforschung*. Vol. III, pp. 106-107, by F. F. Nord and R. Weidenhagen. Leipzig (1934).
- Wohlgemuth, J.
1908 Über eine neue Methode zur quantitativen Bestimmung des diastatischen Ferments. *Biochem. Z.* **9**: 1-9.

RELATION OF WATER ABSORPTION TO THE PHYSICAL PROPERTIES AND BAKING QUALITY OF FLOUR DOUGHS

P. HALTON

The Research Association of British Flour-Millers, St. Albans, England

(Received for publication November 4, 1937)

The term "absorption," when used in connection with the quantity of water added to flour in dough-making, is normally meant to signify that amount of water which in the judgment of the baker will produce a dough of optimum handling and baking quality for the particular flour used.

It is, however, common to find the terms "absorption" and "water content" being used indiscriminately, *e.g.*, "the flour was tested at the three absorptions (water contents) of 15, 16 and 17 gallons per sack,"¹ and it would seem preferable to reserve the term "absorption" for the water content that produces a dough of optimum quality. The question then arises as to how this optimum condition is to be assessed.

The small commercial baker often uses the same quantity of water for all flours and makes but little attempt to judge the best conditions for each delivery, hence the necessity for good tolerance in bread-making flours. This condition of affairs is not universal and it probably does not apply to the larger bakeries where there is often some system of testing before the main bulk is used for bread production.

In test baking, especially where flours milled from individual wheats are examined, the question of water absorption is of great significance since with flours milled from wheats of widely different origin and quality erroneous impressions of their baking qualities may be obtained unless the doughs contain the correct amounts of water.

When the study of the physical properties of doughs was begun in these laboratories it was realised that the water contents at which doughs were to be examined were among the first problems to be solved. Two methods were available—either all flours could be given the same amount of water or else the amount could be varied from flour to flour so that each received its optimum amount. The first method although the simplest was open to the serious objection that the doughs so examined would in many cases be in quite different physical condition from those made from the corresponding flours in the bakehouse. It

¹ One Imperial gallon of water weighs 10 pounds and 1 sack of flour weighs 280 pounds.

therefore appeared desirable to determine by what standards the optimum water content for each dough could be fixed.

The first attack on the problem was an endeavour to determine what were the impressions the baker obtained when making his doughs and how these guided him in assessing the correct amount of water to be used. This proved anything but easy as at this stage there seemed to be a complex mixture of impressions only one of which could be separated and defined, this being the impression of stickiness. The baker disliked a sticky dough and the approach of stickiness appeared to be a limiting factor in assessing the amount of water to be used.

The first attempt at standardising absorption against some physical property of the dough was therefore by measurements of stickiness (Halton and Scott Blair, 1937), but although methods of measuring this property were developed it was not possible to make them precise enough to distinguish with certainty differences in water content that were significant in the bakehouse.

The next attempt was to determine what were the variations in viscosity and elasticity modulus of doughs made from different types of flours when these were tested at the bakehouse absorptions. For this several flours were first examined in the bakehouse on a four-hour system and the optimum water content for each was determined by the baker. Using these absorptions, doughs were made in the laboratory and after four hours' fermentation their viscosities and moduli were measured. The measurements showed that while the viscosities of the doughs differed widely, varying with the strengths of the flours, the moduli differed only to a small degree. This proved to be a most important finding as later experiments showed that the water contents of doughs made from different flours should be adjusted so that the doughs had similar values of elastic modulus; this is necessary not only for the comparison of such flours in the bakehouse, but also for the comparison of the physical properties of the doughs in the laboratory.

In previous papers (Halton and Scott Blair, 1937) the theory was advanced that a high viscosity in dough is desirable since this property largely determines dough stability, *i.e.*, its tendency not to flow out under its own weight, while on the other hand, with modulus a low value was essential for proper expansion of the dough both during fermentation and, more particularly, when the dough is first placed in the oven. The results of experiments were given which showed that changing water content affected both viscosity and modulus in the same way; *i.e.*, increased water content lowered both viscosity and modulus while decreasing the water content raised them both.

Thus, while decreasing the water content had the beneficial effect of raising the viscosity it had at the same time the undesirable effect of

raising the modulus; and for opposite reasons, increasing the water content was desirable from the point of view of modulus but undesirable from the standpoint of viscosity. Thus, optimum absorption is to some extent a compromise between high viscosity and low modulus and

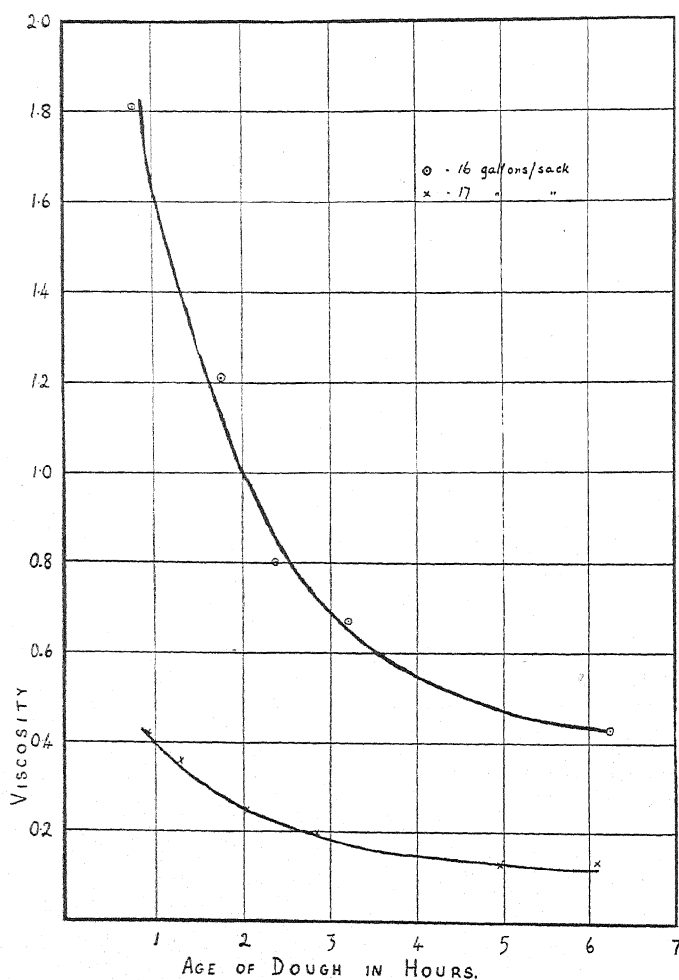


Figure 1. Change in viscosity of dough with age.

is determined by the optimum balance between the two. The balance is, however, not equally adjusted since, as has already been stated, correct absorption corresponds much more nearly to a constant modulus than it does to a constant viscosity.

The viscosity and modulus of a dough are not only affected by changing the water content of the dough, they are also affected by its age. When dough is allowed to stand, either in the presence or absence of yeast, the values of viscosity and modulus both fall with increasing

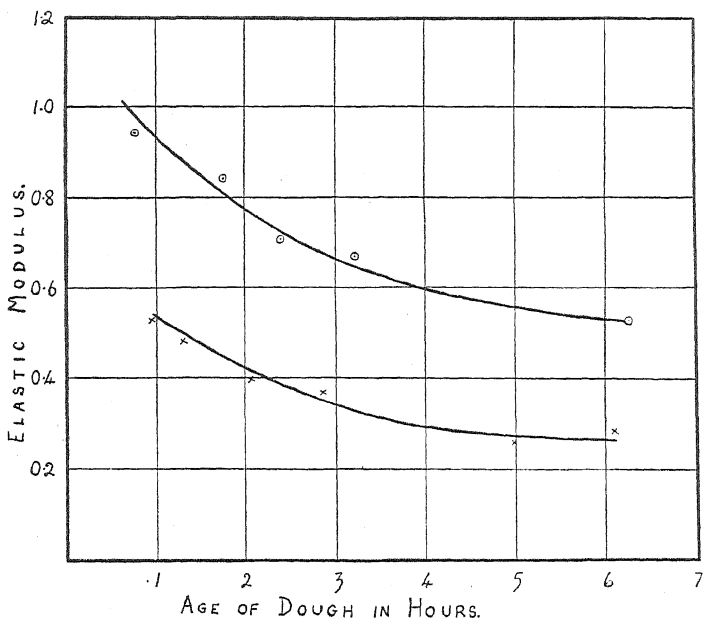


Figure 2. Change in elastic modulus of dough with age.

time.² The decrease is, however, not the same in both since the viscosity decreases more rapidly than the modulus, with the result that the ratio between the two is upset. This is illustrated by the data given in Table I³ where the viscosities and moduli obtained at hourly intervals on two doughs containing 16 and 17 gallons of water per sack respectively, but both made from the same sample of No. 3 Manitoba, are tabulated. From this table it can be seen that the viscosity of the 16-gallon dough fell from 1.66 to 0.43 between the first and sixth hours (that is, by 1.23 or 74%) while the modulus fell during the same interval from 0.94 to 0.53 (or by only 43%). In the case of the 17-gallon dough the viscosity fell from 0.41 to 0.12 (or 71%) and the modulus from 0.53 to 0.26 (or 51%).

² This is complicated by any mechanical treatment the dough may be given during aging, but this does not upset the argument advanced in this paper. The effect of such treatment will be given in a later paper.

³ The hourly figures for viscosity and modulus given in this table are taken from the smooth curves in Figures 1 and 2 where the measured values of η (the viscosity) and n (the modulus) are plotted against the age of the doughs.

TABLE I

VISCOSITY AND ELASTIC MODULUS DATA OBTAINED IN DOUGHS MADE FROM A SAMPLE OF NO. 3 MANITOBA

		Age of dough (hours)					
		1	2	3	4	5	6
16 gal. per sack	Viscosity	1.66	1.01	.68	.54	.46	.43
	(η)						
	Modulus	.94	.78	.66	.60	.56	.53
	(n)						
	η/n	1.8	1.3	1.0	.9	.8	.8
17 gal. per sack	η/n^2	1.9	1.7	1.5	1.5	1.5	1.5
	Viscosity	.41	.26	.19	.15	.13	.12
	(η)						
	Modulus	.53	.42	.34	.30	.27	.26
	(n)						
	η/n	.8	.6	.6	.5	.5	.5
	η/n^2	1.5	1.5	1.6	1.7	1.7	1.8

Note. The above viscosity and elastic modulus measurements should be multiplied by 10^6 and 10^4 , respectively, to express them in C.G.S. units. Therefore the ratios of $\eta : n$ should be multiplied by 10^2 and the ratios of $\eta : n^2$ by 10^{-2} . However, for the sake of simplicity these factors are ignored throughout this paper.

In Figure 3 the viscosity figures given in Table I are shown plotted against the corresponding modulus figures, both in the case of the 16- and the 17-gallon doughs. It will be noticed that the sets of points so obtained both fall on the same smooth curve. This points to the very important fact that the relative change in viscosity and modulus brought about by age is the same as that caused by increased water content. This is also shown up by the following data taken from Table I:

	16 gallons per sack		17 gallons per sack	
	1 hour	6 hours	1 hour	6 hours
Viscosity (η)	1.66	0.43	0.41	0.12
Modulus (n)	0.94	0.53	0.53	0.26
η/n	1.77	0.81	0.77	0.46

From the above it can be seen that for the 16-gallon dough at 6 hours the values for viscosity, modulus and the ratio of the two had fallen to values similar to those obtained at 1 hour with the extra gallon of water. If a dough containing $16\frac{1}{2}$ gallons per sack had been examined the viscosity-modulus curve for this would have overlapped those of the 16- and 17-gallon doughs.

A New Method of Test Baking

In a previous paper (Halton and Scott Blair, 1937) it was suggested that the tolerance of a flour to changing fermentation time was de-

pendent, at least in part, on the change that takes place in the viscosity and modulus, and particularly in the ratio of the two, with changing time. If this is true it would follow from the experiments just quoted that the tolerance of a flour to changing time should be similar to its

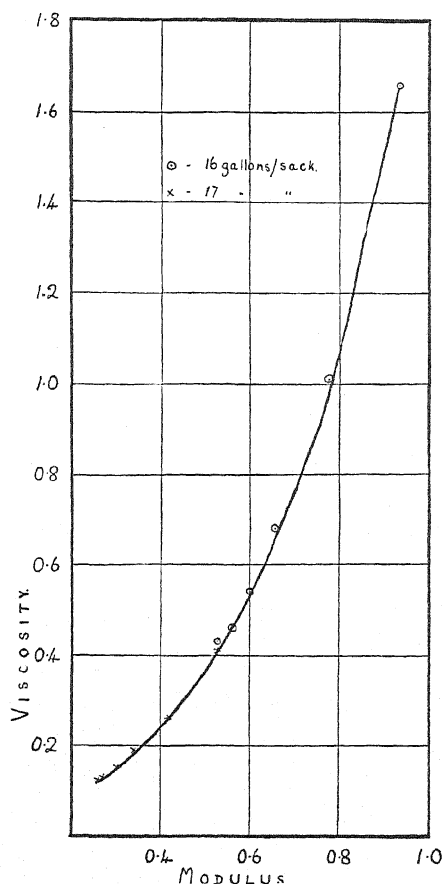


Figure 3. Relationship between viscosity and modulus measurements made on doughs from a sample of No. 3 Manitoba flour.

tolerance to varying water content. This has now been confirmed by tests made in these laboratories over a period of more than a year, and has resulted in a new system of test baking being adopted as our standard procedure.

In our older method (see Fisher and Halton, 1937) it was customary to make at intervals of three-quarters of an hour a series of doughs from the same flour, each having the same absorption. These were fer-

mented and placed in the oven at the same time. As a result a series of loaves were obtained having different fermentation times so that the improvement up to, and the deterioration after, the optimum time were shown up. With a flour of good tolerance a number of similar and good loaves were obtained while when the tolerance was poor one best loaf only was obtained, those having shorter or longer fermentation being inferior. With experience, and a prior knowledge of the type of flour being tested, it became possible to reduce the number of loaves baked and often only three were necessary.

To test the theory that tolerance to time and water were similar two extra loaves were included in the series each having the same fermentation as the middle loaf in the changing time set but one having an extra gallon and the other a gallon less per sack than the standard. This was called "baking the flour on the cross" since, for convenience of comparison, the loaves were arranged for examination as in the following example:

	16 gallons 5½ hours	
15 gallons 4¾ hours	16 gallons 4¾ hours	17 gallons 4¾ hours
	16 gallons 4 hours	

This test has now been made on a large number of flours and it has been found that whenever the tolerance to time is good the tolerance to water is also good and *vice versa*. With Manitoba flours, for example, not only are three good and similar loaves obtained on the varying time system but three good and similar loaves result from changing the water content. With English, or other flours having good tolerance, the centre loaf is usually the best and similar degree of deterioration are shown by increase and decrease of water as by increase and decrease of time. The details of times and water contents are arranged to obtain the best loaf as the centre one, but this is not invariably successful. Sometimes the loaf with the shorter or with the longer fermentation time is the best of the changing time set but in such cases the corresponding loaf with the lowest or with the highest water content is also the best of the changing water series.

In general the loaf with the lowest water content corresponds to the loaf with the shortest fermentation time, while that with the highest absorption matches that with the longest fermentation. Although these corresponding loaves are generally very similar an exact match is not to be expected. This is because the quality of the bread is dependent on other factors besides the viscosity and modulus of the dough. It is, for example, influenced by the rate of gas production, particularly during the final proof. The gas-production curve of a flour approaches linearity over only a comparatively short interval of time with the result that three doughs differing in age by $\frac{3}{4}$ of an hour and $1\frac{1}{2}$ hours respectively may also differ in their rate of gas evolution. Gas production is also influenced by water content of the dough although only to a very small extent, with the result that gassing is a much less variable factor in the "changing water" than in the "changing time" series. The effect of this gas-production factor varies from flour to flour, but in general exact correspondence between any two loaves can only be expected when the rate of gas production as well as the physical properties is the same in both cases.

A difference in rate of gassing may be reflected in a variation in loaf volume which in turn may affect crumb structure. Corresponding loaves may also differ in the character of the crust, particularly in colour. This is because in a series of doughs fermented for increasing lengths of time the amount of sugar used up in gas production increases with time and, even allowing for the continued production by diastatic action, the amount of residual sugar falls. As a result the crust colour, which is largely influenced by the sugar content of the dough at baking, becomes lighter as fermentation increases. With doughs of varying water content but the same length of fermentation the sugar contents at baking are similar and therefore the crust colours are similar.

It is therefore not to be expected that the corresponding loaves in the changing time and changing water series should necessarily be identical. However, it must be pointed out that these differences are only small and in any case do not affect the judgment of baking quality. The object of either system of testing is to determine the optimum quality of bread a flour is capable of producing and its fermentation tolerance. In either system a series of loaves is baked with one variable (either time or water) being altered from loaf to loaf and in this way the conditions for optimum quality are found. Thus with either test the best quality bread is produced somewhere in the series, and since the tolerance to water is similar to the tolerance to time, the same general picture of baking quality results in both cases.

Another possible difference between loaves in the two series can be caused by variation in amount of bakehouse manipulation. While

doughs having varying water contents but the same fermentation time receive the same number of mouldings and knocks back, those with different fermentation times may receive different amounts of treatment.

A third factor which has to be taken into account is the shortness factor, but while the significance of this, as also that of bakehouse manipulation, must be left for future consideration, it can be pointed out that neither materially upsets comparison by the two methods of testing.

In general when 2% of yeast is used loaves baked at $\frac{3}{4}$ -hour intervals of time give a similar picture of flour quality and tolerance to those baked with 1-gallon differences in water content so that, roughly speaking, 1 gallon is approximately equivalent to $\frac{3}{4}$ of an hour. From measurements of viscosity and modulus made with varying time and varying water content it would appear that 1 gallon was equivalent to very much more than $\frac{3}{4}$ of an hour. The reason for this disagreement is not at the moment understood but it must be due to some of the other factors that enter into the determination of bread quality, *e.g.*, oven factors.

As a result of the comparison of the changing time and changing water methods over a considerable period of time, the latter method has been adopted in place of the older method, because of certain advantages the new method has over the old.

With the changing time method the assessment of the correct absorption is in the hands of the baker and this assessment is not easy with certain types of flour. As a result, incorrect deductions are likely to be made from the baking test, errors which are avoided with the changing water method since with the range of water contents used the correct absorption is invariably included among them.

Another advantage of the new method is the smaller variations in gas production that occur compared with the varying time method. This eliminates an error of judgment that is possible when testing poor gassing flours or those that need a long development time on the variable fermentation method. This error is likely to occur because of the falling off in quality of the loaf having the longest fermentation being due, in part or wholly, to a falling off in gassing rather than to a falling off in physical properties. Even when sugar is added gassing is not always prolonged sufficiently to cover the whole range of fermentation times used in the test.

The ideal is that all doughs should gas not merely adequately but at the same rate and this is very nearly so when the water content only is varied.

Optimum Absorption and Optimum Fermentation

Since fermentation time and water content are to a large extent interchangeable in their effects, the terms optimum or correct absorption and optimum or correct fermentation are only relative and not absolute for any particular flour. Thus, if it is desired to produce the best quality bread from a flour at say six hours, the water content of the dough should be less than if the fermentation time were only 3 hours. For similar bread to be made the physical properties of the doughs should be the same at the ends of the two periods, and since in the case of the 6-hour dough softening proceeds for a longer time, this dough requires less water at making than does the 3-hour dough. For the same reason, doughs made from the same flour but having different water contents require different fermentation periods if each is to bake into the same quality bread.

It is thus clear that correct absorption and correct fermentation times are interrelated, and a baker when making his doughs should assess their absorptions not only on the types of flour he is using but also on the length of fermentation he intends to give them.

The Softening of Dough with Age

This has been generally considered as being due to enzyme action (see Jørgensen, 1936), but while probably partly true in the case of doughs containing flour from sprouted wheats or wheats damaged by frost before maturity or attacked by the wheat bug, the experiments quoted earlier in this paper suggest a different mechanism equally as possible in the case of flours milled from sound wheat.

Many protein structures when in a hydrated condition exhibit the phenomenon known as "syneresis." This can be observed to occur with a piece of moist gluten that is covered by an inverted glass beaker to prevent drying, the surface of the gluten becoming gradually covered with drops of water.

Since on aging the viscosity and modulus decrease in the same manner as they do when extra water is added to the dough, it is feasible to suggest that the softening of dough with age is due to syneresis and that the water given out by the protein acts in the same way as added water in "diluting" the dough.

Much interesting information bearing on this softening of dough with age has been obtained and it is proposed to discuss this in a further communication.

Viscosity-Modulus Relationship and Water Content

The experiments quoted earlier in this paper have not only led to a new system of test baking but have also thrown new light on the

importance of the viscosity-modulus relationship as a determining factor in dough quality.

In an earlier report (Halton and Scott Blair, 1937) experiments were described from the results of which a theory was developed concerning the functions of viscosity and elastic modulus in dough behaviour. It was suggested at the same time that the relationship of viscosity to modulus was the best criterion of the strength of a flour since those milled from such wheats as Manitoba had the highest, while English and other soft wheat flours had the lowest value for the ratio of η/n .

It was found that due to the differential effect on viscosity and modulus of age and water content, the simple ratio of η/n was not a constant and comparisons of this ratio for different flours were therefore always made on doughs having the same value for n .

It has since been found that the viscosity-modulus curve, as in Figure 3, is unique for a particular flour; that is, each flour has its own curve which differs in regard to its placings relative to the η and n ordinates from the curve of any other flour. It appeared possible that some other relationship such as η/n^x , where x is some unknown quantity, might be a constant irrespective of dough age or water content.

$$\begin{aligned} \text{If } \eta/n^x &= k \text{ (a constant)} \\ \text{then } \log \eta &= x \log n + k_1 \end{aligned}$$

and the curve obtained by plotting $\log \eta$ against $\log n$ should be linear with a slope of x .

In Figure 4 the logarithms of the corresponding values of viscosity and modulus given in Table I are plotted against one another. The points so obtained while not lying on a straight line, closely fit one, the slope of which is 2. This shows that the ratio η/n^2 has a value which is almost independent of dough age or water content. In Table I the values of η/n and η/n^2 are given for each hour at the two water contents and it can be seen that whereas the value of η/n varied from 1.8 and 0.5, the value of η/n^2 only varied between 1.9 and 1.5.

In the earlier work comparisons of the values of the simple ratio η/n were made at a value of $n = 1$. For each flour a dough was made having a modulus rather greater than 1 and measurements of η and n were made at intervals during the aging of the dough. Curves were then drawn relating η and n to the age of the dough and from these the value of η corresponding to $n = 1$ was read off. The absorptions required to give doughs having this value for modulus were found to be related to those required for the production of optimum quality bread in the bakehouse and experience showed that the values of η/n so obtained agreed satisfactorily with the relative strengths of the flours tested.

The slope of the $\log \eta / \log n$ curve is not exactly 2 for all flours but in the majority of cases varies between 1.8 and 2. With all flours the $\log \eta / \log n$ curve is either a straight line or as in Figure 4 very nearly so, so that in every case η/n^x can be taken as a constant even though the

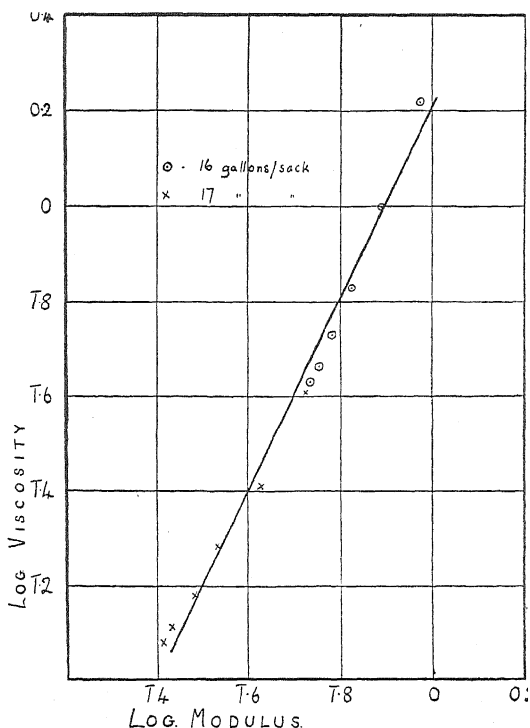


Figure 4. Relationship between the logarithms of viscosity and modulus measurements made on doughs from a sample of No. 3 Manitoba flour.

value of x may vary slightly from flour to flour. This does not upset the convenience of using η/n^x as a measure of flour strength since the value of η/n^x is the same as that of η/n when $n = 1$ and, as has already been stated, the justification for using the latter has been proved by experience.

At the moment our knowledge of the mechanism of the viscous and elastic properties of dough is insufficient to give any reason why the value of x in η/n^x should or should not be the same for all flours, but the fact that for any particular flour η/n^x is a constant shows that η and n are not just two independent variables but that even though they are affected to different degrees by dough age or changing water content, there is some definite relationship between them irrespective of the condition of the dough.

Summary

The effect of aging of dough on the ratio of viscosity to elastic modulus has been found to be the same as that produced by increasing the water content of the dough. An inference from this, which experiment has proved correct, is that the tolerance of a dough to changing water content is the same as its tolerance to changing fermentation time. Based on this a new system of test baking has been devised.

Optimum water absorption and optimum fermentation time are not absolute for any particular flour but are functions of each other.

The value of η/n varies with the age and water content of the dough but the ratio η/n^x , where x has a value of 1.8 to 2.0, is a constant for any given flour and varies only with the quality of the flour used; it is independent of dough age or water content. The value of this ratio is therefore the best relationship of η to n to use as a measure of flour strength.

Acknowledgment

The author wishes to express his thanks to E. A. Fisher, Director of Research, The Research Association of British Flour-Millers, for his interest and suggestions during the progress of this work.

Literature Cited

- Fisher, E. A., and Halton, P.
1937 Studies on test baking. I. The technique and some factors affecting fermentation. *Cereal Chem.* 14: 349-372.
- Halton, P., and Scott Blair, G. W.
1937 A study of some physical properties of flour doughs in relation to their bread-making qualities. *Cereal Chem.* 14: 201-219.
- Jørgensen, H.
1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. *Cereal Chem.* 13: 346-355.

SOME PHYSICAL AND CHEMICAL PROPERTIES OF EGYPTIAN BREAD

CHARLES A. GLABAU and PAULINE F. GOLDMAN¹

Bakers Weekly, 45 West 45th Street, New York, New York

(Read at the Annual Meeting, May, 1937)

In 1935-36 the Metropolitan Museum of Art Egyptian Expedition, headed by Ambrose Lansing and William C. Hayes, carried on excavations in the Asasif Valley on the site of the Ramesside temples and on the upper slopes of the Sheikh Abd el Kurneh Hill, in the interests of Egyptology. After much disappointing work they finally uncovered some valuable material that is discussed by Lansing and Hayes (1937) in a Bulletin of the Metropolitan Museum of Art.

In carrying on clearing work on the west side of Thebes the expedition came to some scant remains of the mortuary temples of Rameses IV, V and VI, and other material that represents the life of the 12th and 25th dynasties. In the debris of the Ramesside temples was found a head representing Amen-Hotpe I, of the 18th dynasty, whose statue had probably once stood in the Avenue of Mentu-Hotpe.

Amen-Hotpe, together with Sen-Mut, Hat-Shepsut, Ra-Mose and Hat-Nufer, plays a prominent part in the expedition's findings which relate to the bread discussed in this paper, as well as other substances with which the authors are not concerned at this time.

It appears that Hat-Shepsut was the reigning queen of the period who had as one of her favorites the architect and builder of the day, Sen-Mut, who was the son of Ra-Mose and Hat-Nufer. In the picture also appears Amen-Hotpe who, so far as can be learned, was related to the king who reigned prior to Hat-Shepsut. She acquired the ruling power, which in all probability belonged to Amen-Hotpe, and since he was a child at the beginning of her reign she apparently had no difficulty in exercising her will upon the people of Egypt.

It also appears that life was carried on very much the same as it is today. Models of granaries and a model of a brewery and a bakery housed in the same building, now in the possession of the Metropolitan Museum of Art, indicate that grain was harvested, threshed, ground into meal and made into various products very much the same as it is in our day. The model of the granary obtained by the expe-

¹ The authors are indebted to Andrew Dingwall for the assistance given and work rendered in the spectrographic work, and to Charles Shillaber for his work in relation to the photomicrographs.

dition shows the Egyptian workers carrying the grain to be measured and stored for future use.

The model of the brewery and bakery shows organized effort which is also quite similar to present-day operations in the arts of brewing and baking. In the brewery the grain was ground and made into a paste or dough and then molded into loaves in very much the same manner as dough is molded in the bakery. This dough was then baked, apparently to obtain gelatinization of the starchy material and coagulation of the protein substance. These loaves, after drying, were ground into a meal which was then mixed with sprouted grain, probably barley, and the mashing process was carried on to obtain conversion of the gelatinized starch into sugar. The mash was then leached by depositing it onto leaves of the sycamore tree that had been spread over gratings. In this way they seemed to have obtained filtration and, at the same time, tannin from the leaves. This wort was then deposited into wooden casks resembling our large fermentation vats, but of much smaller size, where apparently the fluid was fermented. From here it was placed in stone jars and sealed with lids.

The model of the bakery which lies adjacent to the brewery closely resembles the latter; and in the bakery the grain was crushed in the same way and made up into dough. In the operations the dough was then divided into loaves, molded, fermented, and baked in rectangular ovens which in appearance were on the order of our so-called peel-type ovens. Cakes were baked in cylindrical ovens resembling the rotary ovens of today but without a rotating hearth.

Charles Martyn (1927) presents in his record a picture of the foods and culinary utensils used by the ancient Egyptians. The reported history of Egypt, which according to Herodotus was known as Thebes, commences with the reign of Menes, or Menas, who was supposed to have been its first king. He ascended the throne about 2320 B.C. The growth of civilization in Egypt was much more rapid than in any contemporary nation. The early life was devoted to agriculture and the rearing of cattle. Goats were kept for milk. Beef and goose were more generally eaten than any other kind of meat. Lower Egypt used dried and salted birds, such as geese and ducks, as well as many varieties of fish. The Egyptians had frequent banquets and all meat used was freshly slaughtered. They made macaroni and used bronze cauldrons, some alloyed with tin and iron.

The Egyptians were fond of a great variety of cakes and dainty confections. The more elaborate forms of pastry were mixed with fruits and seeds and shaped to represent animals, birds and human beings. The plainer rolls were generally mixed by hand and sprinkled with seeds before baking. At other times they were prepared from a

thinner mixture, well kneaded in large wooden bowls (the feet were often used for this purpose) and then carried in vases to the chief pastry cook who formed it into a sort of macaroni upon a metal pan over the fire, stirring the mixture with a wooden spatula, while an assistant stood ready with two pointed sticks to remove it when sufficiently cooked.

Wine and water were placed in porous jars and fanned until cool. The water was purified by the use of a paste of almonds.

The vegetable kingdom of the Egyptians may be roughly divided into four classes: Trees and shrubs; succulent plants; grains; and various grasses. The most important food-producing trees were the doom and date palms, the sycamore and mokhayp. The wood of the doom palm was used to build boats and the fruit was eaten both ripe and unripe. In the latter state it had about the texture of cartilage and in the former it was similar to the edible portion of the coconut.

Dates were used for making brandy, wine and vinegar. An exhilarating drink was also made from the sap of this tree. The fruit of the sycamore ripened in June and was much esteemed by the Egyptians. The fruit of the mokhayp was used extensively as a medicine and according to Pliny was made into a fermented liquor. Other fruit trees and shrubs include the fig, pomegranate, olive, peach, pear, plum, apple, grapes, etc. The olives were large and fleshy but contained little oil. The juice of the grape was sometimes drunk in the fresh condition but fermentation was usually awaited and the wine stored away in sealed vessels.

The succulent plants included the byblus, papyrus, lotus and nelumbo. Papyrus is no longer found in the country. It grew mainly in marshy districts. The pith of the upper and middle portions was used for paper but the lower portion and the root were considered an edible delicacy.

Wheat and barley were grown in all provinces of the valley and, to a lesser degree, rice, millet, peas, beans, lentils, etc.

The bread and cakes used in the homes of the wealthy were made from wheat flour. Those one degree lower in the social scale used barley meal and the poorer classes ate bread of durrha (*holcus sorghum*) flour.

Grüss (1928, 1929) has reported the composition and physical characteristics of the grain used by the Egyptians in the art of brewing. Nothing of any importance has been found in the literature in regard to the composition of the breads used by the Egyptians of the 18th dynasty other than the study of Whympers (1913) in "The Influence of Age on the Vitality and Chemical Composition of the Wheat Berry." He comes to the conclusion that the baked material was put into the

tombs of the dead for the purpose of furnishing them with refreshment in their journey to the world "beyond the sunset." He also concludes that the baked products were subject to decay due to the fermentable property of the starch and sugar with the result that for the most part only the cellular tissue remained.

This seems to be a general belief and probably for this reason the writers were unable to find in the literature any record of a study having been previously made on these materials. In the case of wheat grains, however, Whympster finds that age has not wrought a very great change. The lowering of the ash content and an increase of acidity are the two marked changes as compared with present wheat but he was unsuccessful in an attempt to germinate the wheat.

The thought may be injected here that to the authors' minds it seems logical that the ancient Egyptians might have deposited these foodstuffs and architectural models into the tombs as a means of preserving for posterity their knowledge and accomplishments.

In cooperation with Lansing samples were obtained of seven products taken from the tombs of Amen-Hotpe, Ra-Mose and Hat-Nufer, which in every respect resemble grain foods of some kind with the possible exception of the product obtained from the tomb of Ra-Mose and Hat-Nufer which appears to be a combination of fruit, largely raisins, which has been charred.

Figures 1 to 3 illustrate the various products obtained in the tombs mentioned.

In Figure 1 Box *A* is apparently a dark breadstuff somewhat resembling in physical characteristics our rye bread. It appears to have been baked in a cylindrical, cone-shaped vessel made of earth. Box *B* is a light, porous product, its physical characteristics being on the order of our present-day honey cake or honey bread. The Egyptians made this product into the images of animals and men. Box *C* is a hollow product having a sort of finger shape similar to our eclairs but made up of grain and possibly other materials. In Figure 2 Box *D* appears to be a residue of mash made up of grain and either raisins or grapes, probably the latter because of the coloring and the character of its composition. This is probably a press residue that remained in the manufacture of wine. It is well known that the Egyptians made wine. Box *E* is a large, somewhat heavy piece resembling bread, with a brownish red color indicating the use of barley or some similar cereal. Box *F* is apparently a grain residue remaining in the process of manufacturing beer. It is largely hulls of grain in which is embedded a leaf of sycamore, as can be seen.

Figure 3 (Box *H*) represents a product on the order of our plum pudding, according to Lansing. It is rather heavy, showing very

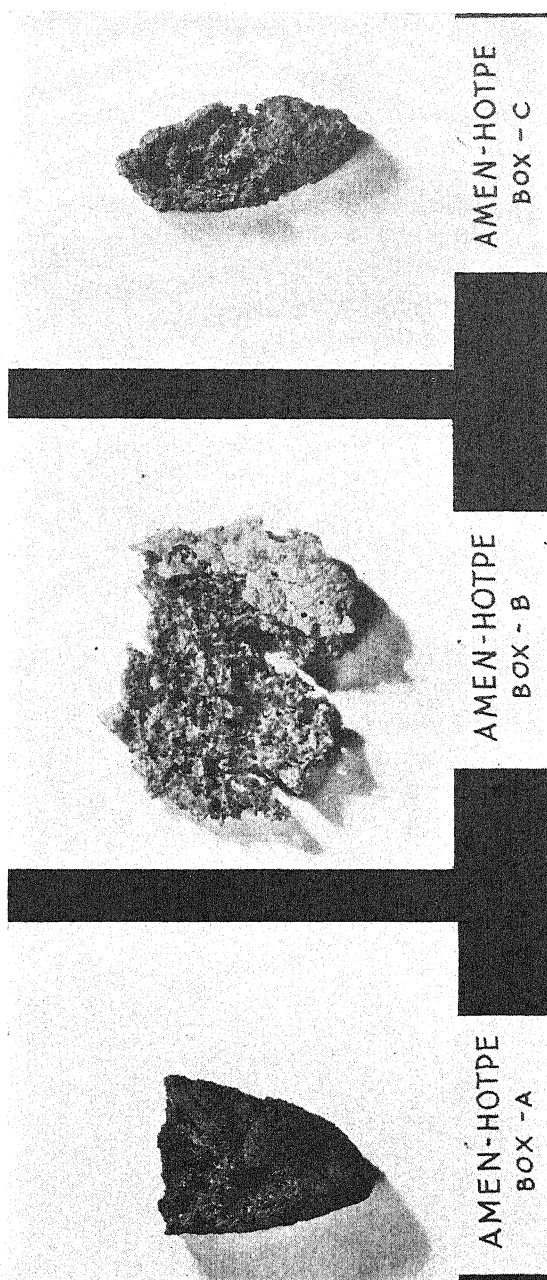


Figure 1

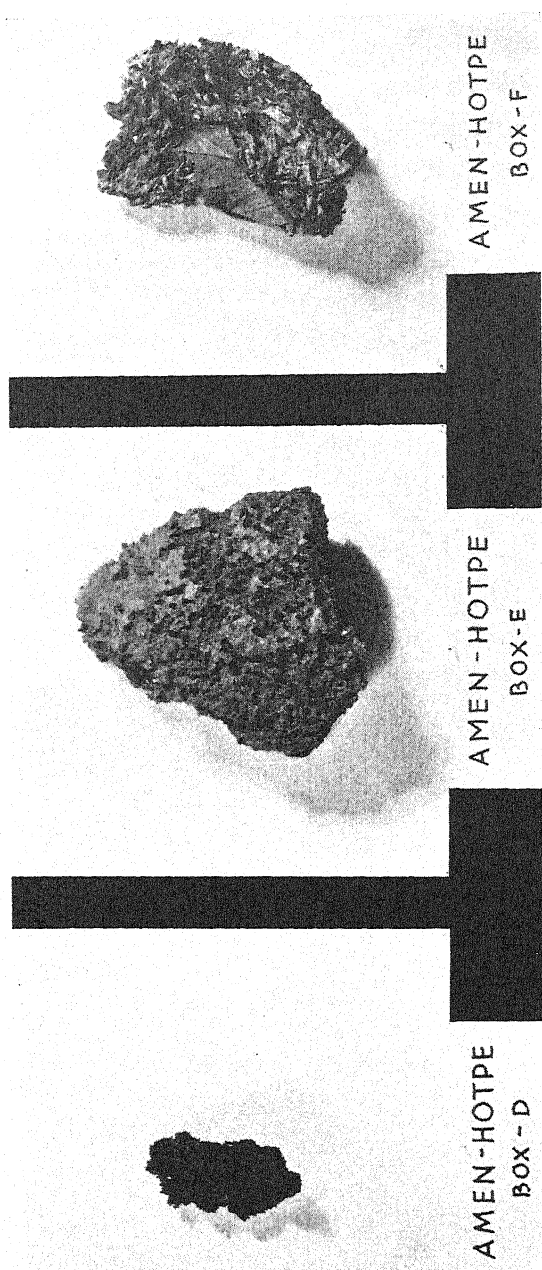


Figure 2

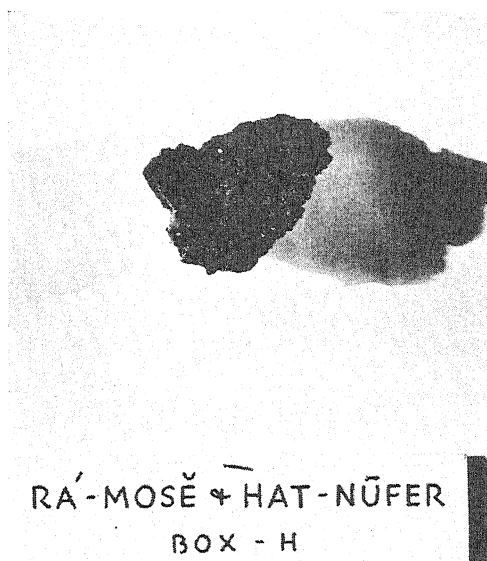


Figure 3

little porosity, is quite charred and has the appearance of being made up of a deposit of fruit with a small quantity of grain to hold it together.

Table I records the analysis of the various products mentioned. It will be seen that the moisture is low in comparison with present-day trends but in keeping with the cakes and so-called hard sweets made by the biscuit and cracker industry of today. Of course, the moisture content obtained by the authors may not be the true moisture content due to the long period of storage.

TABLE I
CHEMICAL ANALYSIS OF EGYPTIAN BREAD

	Amen- Hotpe Box A	Amen- Hotpe Box B	Amen- Hotpe Box C	Amen- Hotpe Box D	Amen- Hotpe Box E	Amen- Hotpe Box F	Ra-Mose and Hat- Nufer Box H
	%	%	%	%	%	%	%
Moisture	7.88	7.90	8.22	9.26	8.24	6.88	9.62
Ash	7.78	4.96	4.20	3.28	3.34	13.30	0.36
Nitrogen	2.40	2.38	2.085	2.705	2.322	3.035	0.825
Protein (N $\times$ 5.7)	14.13	13.57	11.88	15.42	13.25	17.31	4.71
Fat	2.595	2.425	2.595	0.62	1.70	0.48	3.26
Crude fiber	2.26	0.98	2.66	4.02	1.64	16.14	0.60
Salt (Na CL)	1.75	trace	0.627	0.0163	0.288	0.0407	trace
Ash not soluble in HNO ₃	2.32	0.18	0.8	1.60	0.32	7.98	0.02
Dextrin and sugar	68.0	62.0	52.0	40.0	65.0	32.0	30.0
Reducing sugars	—	—	0.450	7.11	0.501	—	21.94
Sucrose	—	—	—	3.42	—	—	0.827
pH value	4.19	5.10	4.47	3.98	4.31	4.94	3.60

The ash is high, especially in some of the products, probably due to their having been baked in earthen vessels and also having come in contact with earth. This can be said of sample *A* in Figure 1. The upper crust had a heavy deposit of what appeared to be ash.

The nitrogen, when multiplied by the protein factor, gives a protein content equal to that of the protein of cereals today. The fat, too, is in keeping with some of our yeast-leavened goods. The fibre indicates that whole grain was employed in the manufacture of the substances.

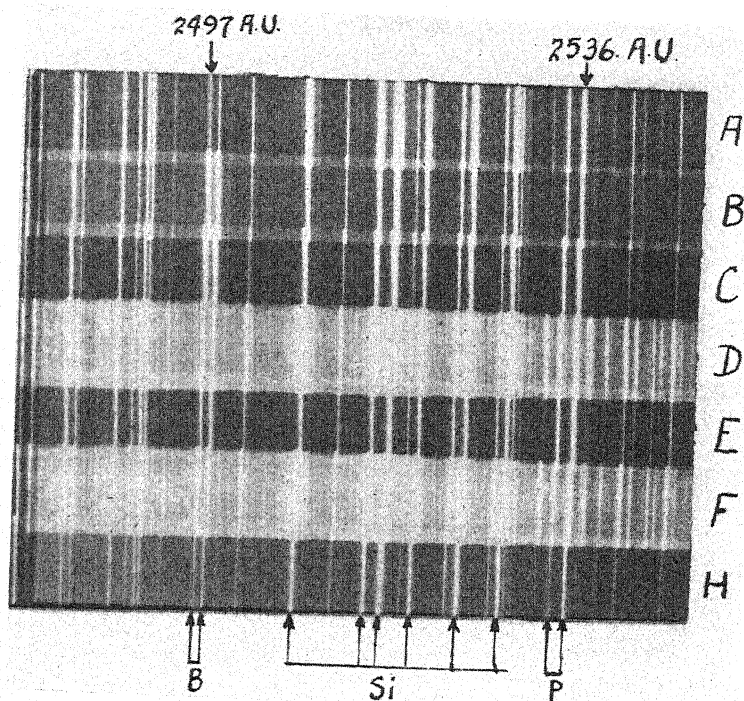


Figure 4

The chlorine, calculated as sodium chloride, varies considerably from just a trace to 1.75%. The ash in sample *A* (Figure 1) and in sample *F* (Figure 2) is very high.

The authors carried on some work to determine the quantity of sugar present and, in an effort to conserve the sample, came to the conclusion that it would be best to work with a polariscope. It was soon discovered that only two samples gave a very slight starch reaction, and in the polarization work as well as in the gravimetric work

large quantities of dextrin were found in each sample. Consequently it was necessary also to run a sugar determination gravimetrically. Since the polarization was much higher than the quantity of sugar obtained by direct analysis, and the qualitative test indicated dextrin,

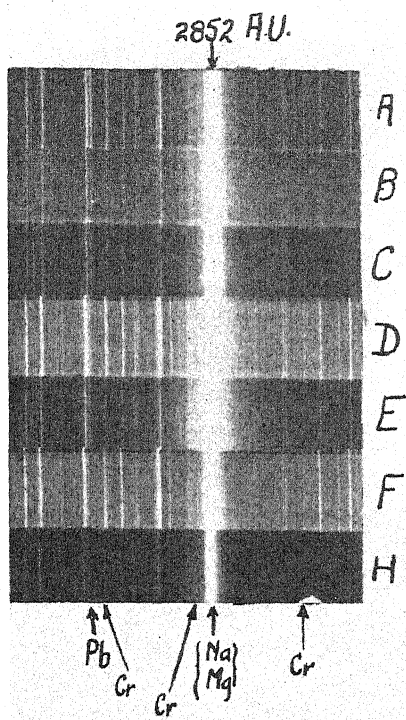


Figure 5

the figure obtained was designated as dextrin and sugars. This indicates that the starch had been converted into dextrin, which is quite possible as the samples had been stored since 1494 B.C.—almost 3,500 years.

All of this material is of the 18th dynasty, during the reign of Queen Hat-Shepsut, the date having been fixed from inscribed objects found in the tombs of Ra-Mose and Hat-Nufer. The conversion of the starch into sugar by heat over a long period of time is easy to assume. The temperature within the tomb where this material was discovered is somewhere around 110° F., and in resting over a period of almost 3,500 years it is quite possible that all of the starch material would become dextrinized.

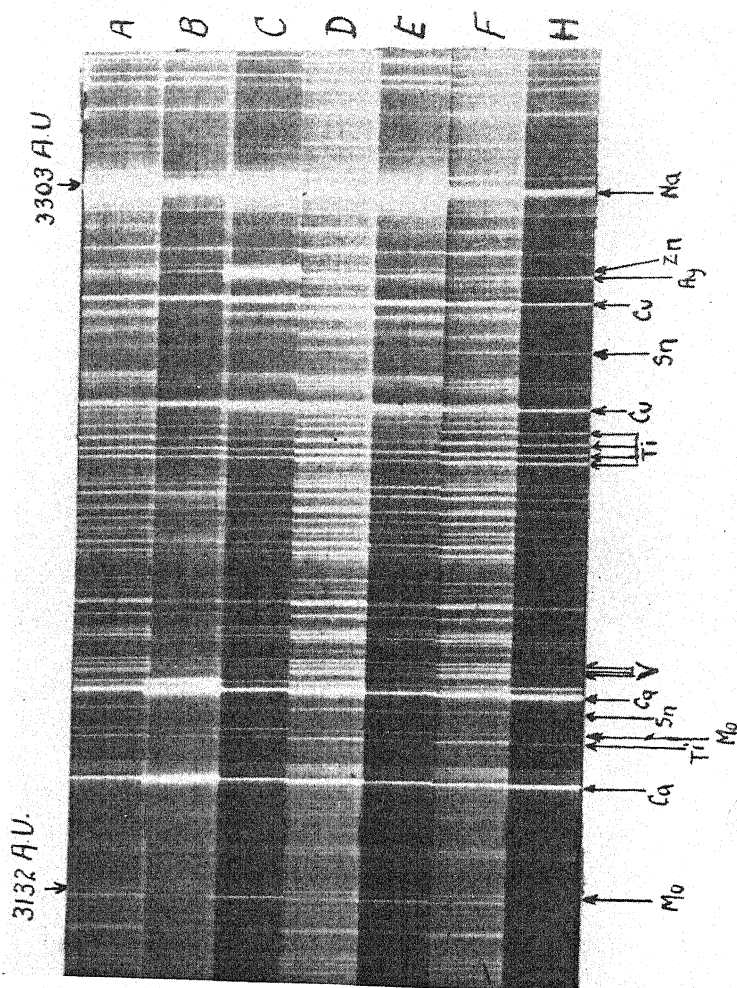


Figure 6

The dextrinization of starch has been reported by Bouillon-Lagrange (1904) and he published his method of dextrinization of starch in 1911.

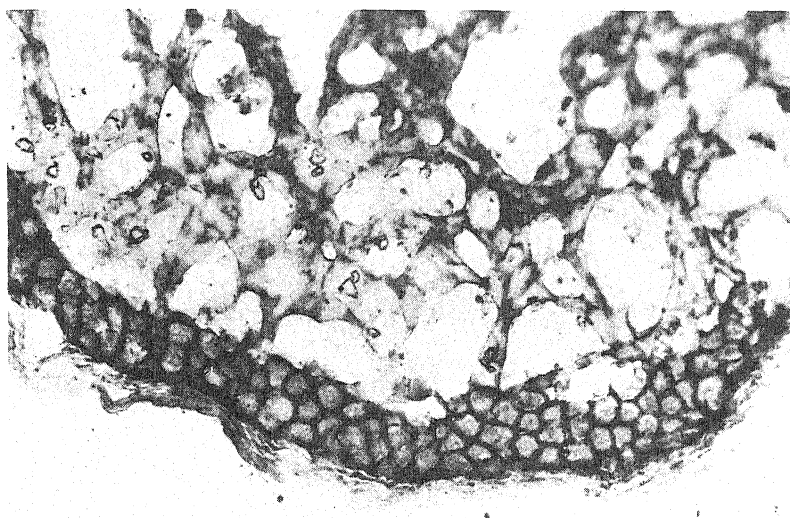


Figure 7

The method used in making these various determinations follows:

Moisture: Determined according to the A. A. C. C. method for baked cereal products.

Ash: Determined according to official A. A. C. C. method for baked cereal products.

Nitrogen: Determined according to the A. A. C. C. method for determining nitrogen in baked cereal products.

Protein: Percentage of protein obtained by the use of the factor 5.7.

Fat: Determination was made following the A. A. C. C. ammoniacal alcohol method for baked cereal products.

Crude fibre: Determined in accordance with the official A. A. C. C. method for cereal products.

Chlorides: Determined by following the A. A. C. C. method for determining sodium chloride in the ash of cereal foods.

Dextrins; Sugars: So designated due to the fact that a qualitative dextrin determination on the products gave a positive result, and in the attempt to determine sugars by polarization according to the A. A. C. C. method for polarizing sugars the results obtained were too high to be indicative of the sugars alone.

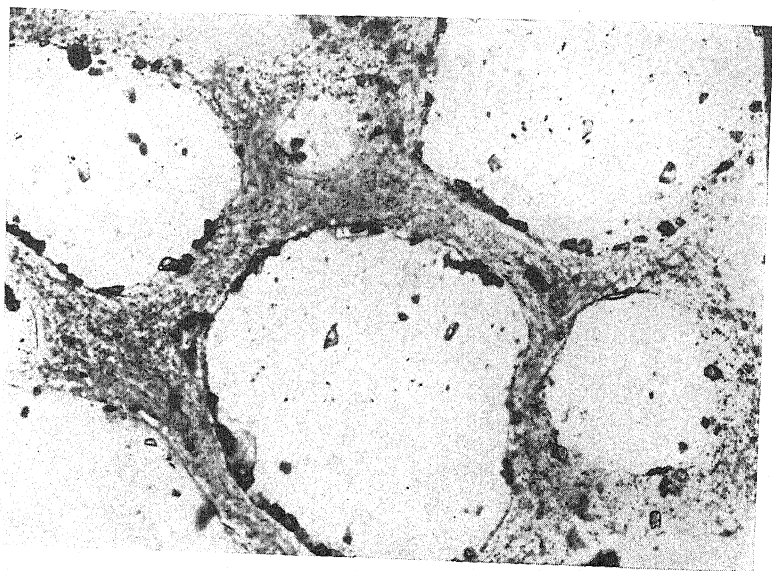


Figure 8

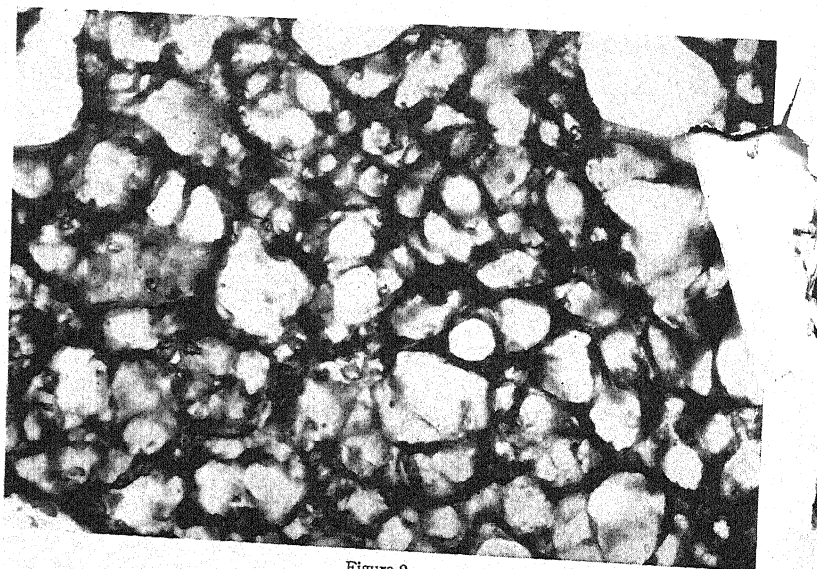


Figure 9

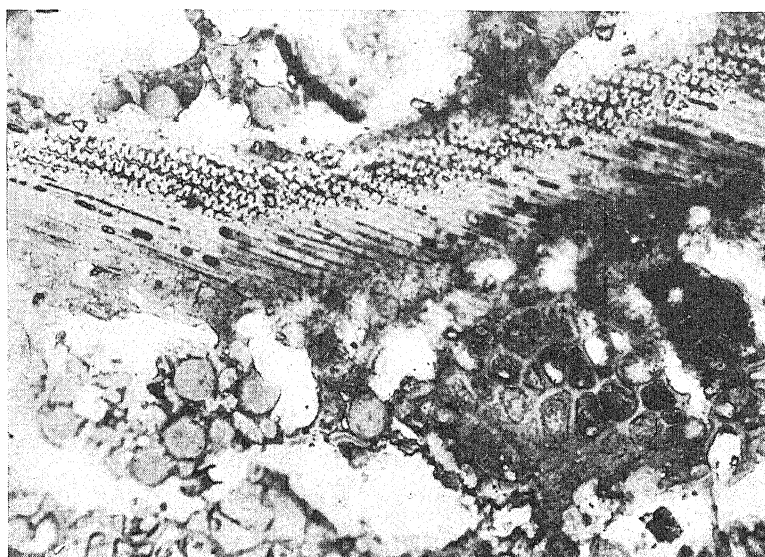


Figure 10

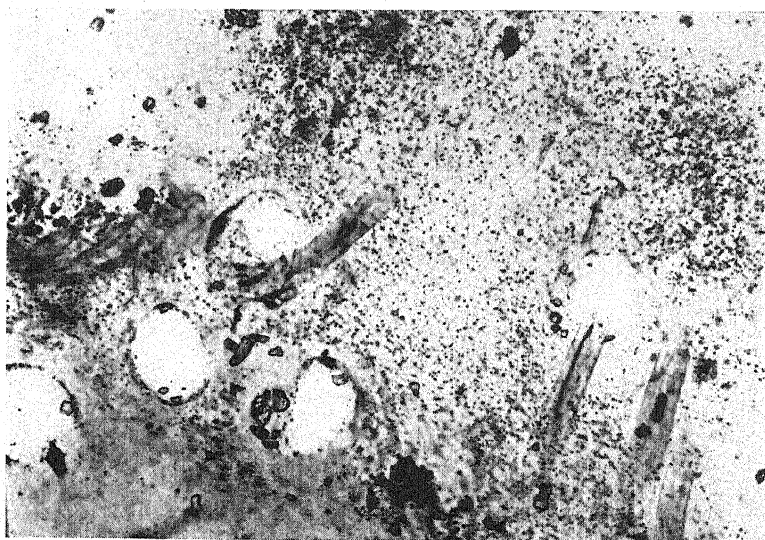


Figure 11

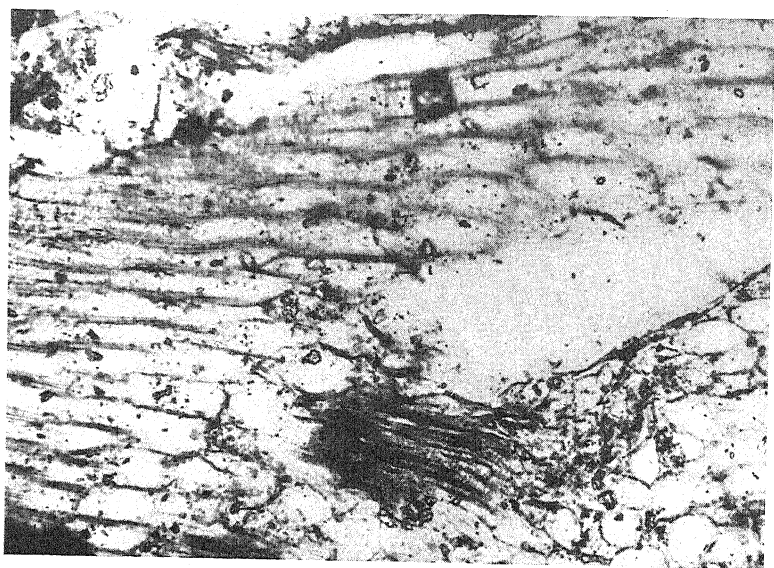


Figure 12

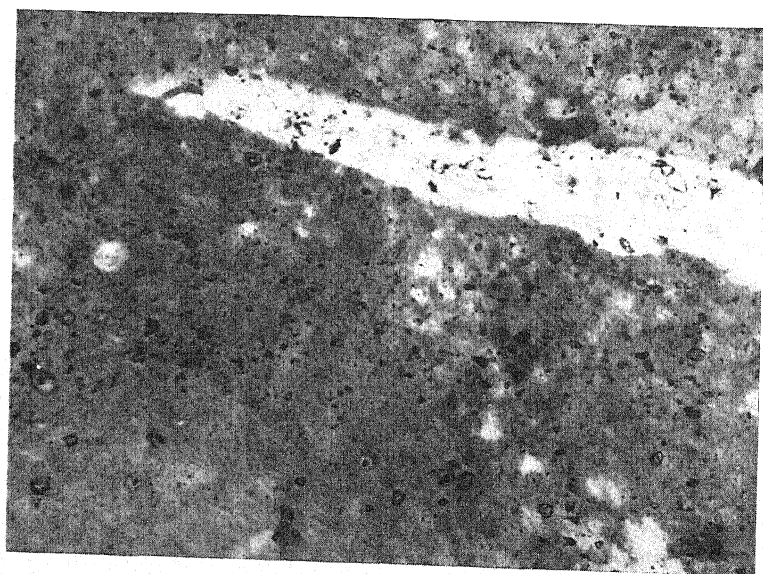


Figure 13

Reducing Sugars and Sucrose: Determined according to the Munson-Walker method for determining these on cereal foods.

In addition to making a chemical analysis, a spectrograph of the minerals contained in these breads and cakes was also obtained. Silver, boron, copper, tin and lead were found. Samples *A*, *D* and *F* contained rather heavy deposits of lead. Of course, lead is also present in the others.

Figures 4 to 6 represent a spectrum of the ash of these materials. In addition to the spectrograph, photomicrographs were made of portions of the structure of the products, as shown in Figures 7 to 13. Difference in the structure of the photomicrographs will be noted. Figure 7 is particularly interesting in that it shows, in the lower portion, a micrograph of the crust structure and adjoining this is the crumb structure. Considerable fibre was noticeable in these various samples, excepting sample *H* which under the microscope resembled a resinous material (see Figure 13). There was a continuous field in observing this material under the microscope.

Literature Cited

- Bouillon-Lagrange
 1904 A comprehensive survey of starch chemistry. (R. P. Walton, Vol. 1, 1928.) See also Bulletin de Pharmacie, Tome 3, pp. 395-398, Paris, 1911.
- Grüss, Johannes
 1928 Zeitung für Brauerei, p. 1123, October 7.
 1929 Ibid., p. 8275, March 10; p. 517, May 12; p. 679, June 23.
- Lansing, Ambrose, and Hayes, W. C.
 1937 The Egyptian Expedition, 1935-36. Bulletin of the Metropolitan Museum of Art (New York). Sec. 2, Jan. 1937.
- Martyn, Charles
 1927 Foods and Cooking Utensils of the Ancients. (Compiled from standard historical works.) Caterer Publishing Co., New York.
- Whymper, Robert
 1913 The influence of age on the vitality and chemical composition of the wheat berry. Knowledge 36. New series, 10, 1913.

PHOTOELECTRIC SPECTROPHOTOMETRY APPLIED TO THE QUANTITATIVE ANALYSES OF CAROTENOID AND CHLOROPHYLL PIGMENTS IN TERNARY AND QUATERNARY SYSTEMS¹

ELMER S. MILLER

Department of Botany, University of Minnesota, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1937)

Recently, spectral analyses have been applied extensively to binary mixtures by Kuhn and Smakula (1931), Zscheile (1934), Miller (1934), Dingwall and Thomson (1934), and Van der Hulst (1935). This paper concerns the quantitative analyses of the carotenoid and chlorophyll pigments in ternary and quaternary systems by photoelectric spectrophotometry. For convenience, the paper is divided into (a) ternary mixtures of known composition, (b) a simple test case of unknown ternary mixtures, (c) known quaternary mixtures, and (d) unknown quaternary mixtures and the standardizing of spectral analytical method. The apparatus and method of measuring absorption coefficients have been described in detail by Miller (1937). Additional details regarding the construction, calibration, and use of a photoelectric spectrophotometer in chemistry have been published recently by Hogness, Zscheile, and Sidwell (1937).

Ternary Systems

Following the convention suggested by Miller,² the effective absorption at λ' for a ternary mixture is expressed by equation 1.

$$\alpha_T^{\lambda'} = \alpha_1^{\lambda'} \left(\frac{C_1}{C_T} \right) + \alpha_2^{\lambda'} \left(\frac{C_2}{C_T} \right) + \alpha_3^{\lambda'} \left(\frac{C_3}{C_T} \right). \quad (1)$$

The absorption spectra reported by Miller (1935a) were employed as standards and a guide in selecting suitable wave lengths of light to be employed in analyses. As a test case of a very simple ternary system, mixtures of known composition were employed. For this, lycopene, beta carotene, and leaf xanthophyll³ were selected because the posi-

¹ Presented at the twenty-third annual meeting of the American Association of Cereal Chemists at Minneapolis, May 27-28, 1937.

² The writer wishes to acknowledge and express thanks to A. E. Sidwell, Research Associate, Department of Chemistry, University of Chicago, for aid in making some of the calculations and tissue extractions employed in this study on ternary systems.

³ Strain (1936) has shown that leaf xanthophyll consists of several isomers. Since the leaf xanthophyll employed in these mixtures was identical with that used by Miller (1935a), the use of this standard is permissible. In the subsequent paragraphs the method of standardizing the xanthophyll analyses is described. Since the author has stated the absorption coefficients at the various wave lengths that were employed as standards, it is possible to make the necessary correction for absolute analyses should the reference xanthophyll curve be too high or too low.

tions of their absorption bands occur at different wave lengths. From known stock solutions, aliquot portions were measured volumetrically and a series of mixtures was made so that in a particular series each component formed 12.5, 25.0, 33.3, 50.0, and 75.0% of the total solute. All measurements were recorded the same day these ternary mixtures were made. The analytical results of this experiment are summarized in Table I.

TABLE I
SPECTRAL ANALYSES OF TERNARY MIXTURES CONSISTING OF BETA CAROTENE,
LEAF XANTHOPHYLL AND LYCOPENE

	Known composition in per cent				
	12.5	25.0	33.3	50.0	75.0
Composition found by spectral analyses at wave lengths	Composition in terms of lycopene				
5150 A.U.	12.0	24.7	33.0	49.6	75.2
5200 A.U.	11.9	25.4	33.9	49.8	75.7
Average	11.95	25.05	33.45	49.7	75.45
	Composition in terms of leaf xanthophyll				
4600 A.U.	12.7	23.6	33.5	50.3	75.9
4750 A.U.	13.2	25.2	34.1	51.1	76.0
Average	12.95	24.4	33.8	50.7	75.95
	Composition in terms of beta carotene				
4575 A.U.	13.6	24.2	34.0	50.6	74.3
5000 A.U.	12.0	24.4	32.9	49.3	74.5
Average	12.8	24.3	33.45	49.95	74.4

As an additional check that the correct analyses were obtained (Table I), λ s 5200 and 4450 A.U. were found satisfactory for measuring total lycopene and the total concentration of the other two components—treated as a binary system.

Analysis of Unknown Ternary Mixtures

An extract of ripe tomatoes was employed to further test the application of photoelectric spectrophotometry on unknown ternary mixtures. The tomato tissue was extracted by the method as outlined by Miller (1935). These analyses are reported in Table II.

TABLE II
SPECTRAL ANALYSES OF LYCOPENE, LEAF XANTHOPHYLL, AND BETA CAROTENE IN
RIPE TOMATOES
(Analyses expressed in per cent on green weight basis)

Pigment Wave length employed A.U.	Lycopene		Beta carotene 4950	Leaf xanthophyll	
	5200	5150		4750	4600
Sample					
1	0.00221	0.00223	0.000763	0.000380	0.000390
2	0.00227	0.00226	0.000752	0.000375	0.000380

The averages of the values reported in Table II were substituted in equation 1 for a wave length at which α_1 , α_2 , and α_3 are of different magnitude. When this was done for 5000 A.U., the calculated value for α_T agreed within 1% with the experimentally determined value. This offers a rigorous check as to the accuracy of the analyses reported in Table II. Thus, it is possible to analyze ternary mixtures, of which neither the total concentration nor composition is known with an error of approximately 2%.

Quaternary Systems

Since ether was employed as the solvent for the final extractions of plant tissues, it was selected as the solvent for quaternary systems. The absorption spectra of chlorophylls *A* and *B* as determined by Zscheile (1934)⁴ were employed as standards. For leaf xanthophyll and beta carotene the respective absorption spectra were measured in diethyl ether from which the absorption curves (Figure 1) were calculated. In ether both of these pigments have an alpha of 218.5 at λ 4775. The alphas at λ 4850 and λ 4900 are 195 and 158.5 respectively for beta carotene and 139.5 and 78.0 respectively for leaf xanthophyll.

As described for the ternary mixture, aliquot portions were taken from the stock solutions of chlorophylls *A* and *B*. Leaf xanthophyll and beta carotene and mixtures of known composition were made. Spectrophotometric analyses of these mixtures show that the following analytical procedure is feasible:

(1) Total chlorophyll may be determined by employing λ 6450 A.U. with a $\pm 2.5\%$ error. The absorption coefficients for both the chlorophylls are identical at this wave length.

(2) The error was 1% or less when chlorophyll *A* concentration was determined by λ s 6600 and 6700 A.U. Proper analytical checks were possible in determining this component by employing two or more different wave lengths.

(3) Figure 1 illustrates the relative absorption by the plant pigments per 100 parts of chlorophyll *A*. This distribution is for extracts of corn and barley tissues. Figure 1 also illustrates that λ 4775 A.U. permits the measurement of total carotenoids; yet at this wave length the absorption by the chlorophylls is low—only 5 to 6%. The magnitude of the correction may be calculated after total chlorophyll is determined by λ 6450 A.U. Wave lengths 4850 and 4900 A.U. were employed for the analyses of beta carotene (error $\pm 2.0\%$) by use of equation 2.

$$\alpha_T = (\alpha_1 - \alpha_2) \frac{C_1}{C_T} + \alpha_2. \quad (2)$$

⁴ On a sample of chlorophyll with the proper magnesium analysis, the author measured the absorption coefficients and found the spectra to be similar to those reported by Zscheile (1934a).

Additional data are presented under analyses of unknown quaternary mixtures, on the validity of employing λ s 4775, 4850, and 4900 A.U. in measuring beta carotene and leaf xanthophyll (total xanthophyll).

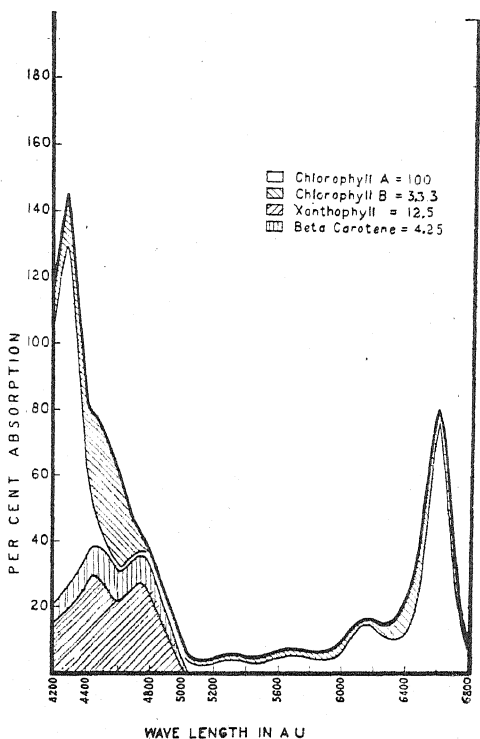


Figure 1. The relative absorption of light by total leaf xanthophyll, beta carotene, and chlorophyll *B* per 100 parts of chlorophyll *A*.

(4) Since total chlorophyll as measured by λ 6450 A.U. contains $\pm 2.5\%$ error, it becomes 8 to 15% error in chlorophyll *B* analyses (by difference) because the concentration of chlorophyll *B* is so low. Thus, the large band of chlorophyll *B* at λ 4525 A.U. was employed for analysis of this component. Data in Figure 1 show that the absorption by the carotenoids at λ 4525 A.U. is approximately $8 \pm 0.5\%$ more than at λ 4775 A.U. Hence, by subtracting the carotenoid absorption at λ 4525 A.U. from α_T 4525 A.U. the difference is due to chlorophyll *B* absorption. This proved possible on the *known solutions* and the error in the chlorophyll *B* analyses was reduced to $\pm 0.7\%$ (or less).

Briefly, the analyses of quaternary systems may be performed by employing λ 4525 A.U. for chlorophyll *B*, λ s 6600 and 6700 A.U. for chlorophyll *A* and λ 6450 A.U. for estimating total chlorophyll. Wave

lengths 4775, and 4850 and 4900 A.U., were used for total carotenoids and beta carotene respectively (with proper corrections).

Unknown Quaternary Mixtures

The above method of analyses has been further standardized to permit the quantitative measurement of chlorophylls *A* and *B*, leaf xanthophyll and beta carotene in barley and corn tissue extracts (in ether). The absorption by the carotenoids at λ 4525 A.U. was compared to that at λ s 4700, 4775, 4780, 4790, and 4800 A.U. These measurements were made on saponified extracts of barley and corn tissue. These data are summarized in Table III.

TABLE III

THE RATIO BETWEEN β_T^* FOR WAVE LENGTH 4525 A.U. AND β_T FOR WAVE LENGTHS 4770, 4775, 4780, 4790 AND 4800 A.U. FOR TOTAL CAROTENOIDS IN ETHER AFTER SAPONIFICATION

Series	A.U.	β_T for λ 4525 A.U.	
		$\beta_{T_1} \dots$	$\dots \beta_{T_5}$
Corn tissue extract			
$\beta_{T_1} \dots$	4770		1.145
$\beta_{T_2} \dots$	4775		1.16
$\beta_{T_3} \dots$	4780		1.18
$\beta_{T_4} \dots$	4790		1.22
$\beta_{T_5} \dots$	4800		1.25
Barley tissue extract			
$\beta_{T_1} \dots$	4770		1.07
$\beta_{T_2} \dots$	4775		1.09
$\beta_{T_3} \dots$	4780		1.106
$\beta_{T_4} \dots$	4790		1.13
$\beta_{T_5} \dots$	4800		1.16

$$^* \beta_T = \alpha_T C_T.$$

The same ratios for $\lambda_1 \dots \lambda_5$ were obtained on tissue extracts of etiolated corn and barley as reported in Table III for mature plants. The ratio for λ 4775 A.U. for barley tissue agrees with the ratio presented data in Figure 1; but the ratios for corn tissue extracts are higher, due to the presence of different xanthophyll isomers. Analyses made on corn tissue extracts before and after the removal by saponification⁶ are summarized in Table IV.

Data presented in Table IV illustrate that by correcting for total chlorophylls it is possible to determine accurately the total carotenoid concentration in the presence of the chlorophylls.

A similar test was made for beta carotene analyses. When the chlorophylls and total xanthophyll were removed (by saponification and 92% methanol-ligroin partition respectively) the amount of beta

⁶ A short saponification was employed. The time factor was 2 minutes. The saponification was carried out by adding the hot MeOH-KOH solution to the extract with the minimum amount of shaking. Moore (12) has shown that long saponification and vigorous shaking may cause chemical changes and oxidation.

carotene present (in 20% diethyl ether and 80% ethanol) agreed with the amount as determined by λ 4850 A.U. in the quaternary mixture. The maximum variation was 2.5 to 3.5% between the two methods for a series of five samples. The error is greatest for the beta carotene component because of its low concentration.

TABLE IV

A COMPARISON OF TOTAL CAROTENOID ANALYSES BEFORE AND AFTER THE REMOVAL OF THE CHLOROPHYLL (BY SAPONIFICATION)

Series	Total carotenoids analyzed in ether in the presence of chlorophylls <i>A</i> and <i>B</i>	Total carotenoids analyzed after saponification of the chlorophylls in 20% ether and 80% ethanol
	%	%
1	0.07630	0.07572 $\pm$ 0.00071
2	0.01515	0.01483 $\pm$ 0.00015
3	0.01385	0.01405 $\pm$ 0.00024
4	0.01495	0.01470 $\pm$ 0.00015
5	0.01310	0.01336 $\pm$ 0.00013
6	0.01305	0.01331 $\pm$ 0.00011
7	0.01105	0.01093 $\pm$ 0.00010
8	0.00860	0.00872 $\pm$ 0.00009

The large error (3 to 4%) for beta carotene is unavoidable because it (in leaf tissue extracts) represents only 3 to 4% of the total pigment content. The error in the determination of chlorophyll *A* and leaf xanthophyll is about 1.0%, and 2% or less for chlorophyll *B*.

Burr and Miller (1937) in following the development of the chlorophylls in young etiolated barley and corn, obtain chlorophyll *A*/chlorophyll *B* ratios as large as 100. Under approximately the same experimental conditions Inman (1937) has obtained similar chlorophyll ratios.

Summary

1. Photoelectric spectrophotometry has been satisfactorily employed to analyze ternary and quaternary mixtures with less than 2% error, providing each component is present in sufficient concentration to form more than 10% of the total pigment content.

2. Unknown ternary mixtures obtained from tomato tissue may be analyzed satisfactorily. By employing two or more wave lengths per sample per component, lycopene, leaf xanthophyll, and beta carotene analyses on duplicate samples agree with an error of only 2%. Part of this error is due to the heterogeneous distribution of the pigments in the tomato tissue.

3. Chlorophyll ratios determined by this physical method confirm those reported by Inman, who employed a chemical method.

4. The method permits ternary and quaternary mixtures to be analyzed with almost the same degree of rapidity as those solutions containing single component pigment.

5. Quantitative analyses can be made with the minimum amount of photo-oxidation—less than the experimental error.

Literature Cited

- Burr, George O., and Miller, Elmer S.
1937 Chlorophyll ratios and photosynthesis. (In press.)
- Dingwall, A., and Thomson, J. C.
1934 The preparation of pure eleostearic acids from Chinese wood oil. *J. Am. Chem. Soc.* 56: 899-903.
- Hogness, T. R., Zscheile, F. P., Jr., and Sidwell, A. E., Jr.
1937 Photoelectric spectrophotometry. *J. Phys. Chem.* 41: 379-415.
- Van der Hulst, L. T. N.
1935 The application of absorption spectra to fatty oil research. *Rec. des trav. chim.* 54: 639-650.
- Inman, Ondess L.
1937 The ratios of chlorophyll *A* and *B* and the mechanism of photosynthesis. *Science* 85: 52.
- Kuhn, Richard, and Smakula, Alexander
1931 Spektrophotometrische analyse des Eidotterfarbstoffes. *Z. physiol. Chem.* 197: 161-166.
- Miller, Elmer S.
1934 Application of quantitative spectral analyses to binary mixtures of the common carotenoids. *Plant Physiol.* 9: 681-684.
1935 A rapid and accurate quantitative method for the determination of the common carotenoids; analyses of beta carotene and leaf xanthophyll in thirteen plant tissues. *J. Am. Chem. Soc.* 57: 347-349.
1935a Improved methods for the purification of the common carotenoids and the quantitative measurement of their absorption spectra. *Botan. Gaz.* 96: 447-467.
1937 A precise method with detailed calibration for the determination of absorption coefficients; the quantitative measurement of the visible and ultraviolet absorption spectra of alpha carotene, beta carotene, and lycopene. *Plant Physiol.* 12: 667-684.
- Strain, H. H.
1936 Leaf xanthophylls. *Science* 83: 242.
- Zscheile, F. Paul, Jr.
1934 A quantitative spectrophotoelectric analytical method applied to solutions of chlorophylls *A* and *B*. *J. Phys. Chem.* 34: 95-102.
1934a An improved method for the purification of chlorophylls *A* and *B*; quantitative measurement of their absorption spectra; evidence for the existence of a third component of chlorophyll. *Botan. Gaz.* 95: 529-562.

THE COLLOIDAL BEHAVIOR OF FLOUR DOUGHS.

II. A STUDY OF THE EFFECTS OF VARYING THE FLOUR-WATER RATIO¹

MAX C. MARKLEY AND C. H. BAILEY

Division of Agricultural Biochemistry, University Farm, St. Paul, Minnesota

(Received for publication March 28, 1938)

The term absorption has been used in several different ways in measuring and describing the properties of flour. Not uncommonly it implies the ratio of water to flour required to bring the dough to a definite and more or less arbitrary level of relative plasticity or viscosity. In a limited number of cases it is estimated from the observed plasticity of dough prepared with a definite or constant ratio of flour to water. The authors prefer to regard absorption as the proportion of water to flour which results in dough, and hence in bread, of optimum properties. Thus cognizance is taken of possible changes in dough properties during fermentation. At best, the absorption thus defined is not an absolute value but will vary with any particular lot of flour, depending upon the dough formula, fermentation program, and type of bread desired.

A variety of methods has been employed for determining flour absorption, the details of which were conditioned by the individual's definition of absorption, and the mechanical facilities available. Before testing instruments were employed, the relative mobility of dough was observed by the feeling, or force exerted by the hand in effecting a plastic flow of the dough. Bakers became very skillful in judging doughs by the sense of touch. Then simple viscosimeters made their appearance in a number of forms, that described by Jago and Jago (1911) being typical. In the instance of this instrument, a quantity of dough was placed in a cylinder provided with a standard aperture at the bottom. A unit force was applied by means of a weight at the top of the dough column, and the rate of flow through the plastometer opening was measured.

Briggs' (1918) "Perfekdo" device was somewhat similar. This employed atmospheric pressure, applied by moving a standard glass tube up to the dough surface, reducing the pressure at the other end of the tube by a suction pump and observing the rate of flow of the

¹ Paper No. 1517, Scientific Journal Series, Minnesota Agricultural Experiment Station.

dough into the graduated tube. An elaboration of this general method, but refined to permit of calculating the results in absolute units, was employed by St. John and Bailey (1929).

Rejto's ductility machine, described by Kosutany (1907), enabled him to measure the relative force required to extend doughs containing varying proportions of water. In a series of doughs prepared from the same flour but with varying proportions of water, ranging from 45 to 50% of the flour, the "Zugkraft" was determined and recorded (Kosutany, p. 262) as follows:

Water, %	Zugkraft, g.
45	937.75
46	852.50
47	775.00
48	676.73
49	596.75
50	530.10

If the logarithms of water used, and of Zugkraft be plotted, the points fall nearly on a straight line.

Harrell (1927) designed a penetrometer for use with large doughs in the bakery while van der Lee (1929) employed the same general principle in a laboratory instrument.

Chopin's (1921) extensimeter afforded a measure of the force required to extend a dough surface, which was recorded by a pressure gauge. He observed that this force was related to the proportion of water in the dough. Bailey and LeVesconte (1924) investigated the applicability of this device to American flours. Several similar devices including the Buhler "Comparator" (see Kozmin 1936) and the Borasio and de Rege Pneumodynamometer (1934) could doubtless be employed in like manner.

Swanson and Working (1933), and Working (1934) reported on the use of a centrifuge in measuring water absorption and Fifield (1933) described his experiences with the same procedure. Halton and Scott Blair (1936) departed rather widely from the earlier practices; they added water to the point where the dough possessed a standard stickiness as measured by the force required to overcome the adhesion of a metal weight to the surface of the dough.

Another and singularly useful criterion of relative dough mobility is afforded by devices which measure or record the force required to move the arms or blades of a mixing machine through a dough. This method was employed by Bailey (1930) in measuring the work-input (in watt hours) of a dough mixer, the blades of which were rotated a unit number of times. St. John and Bailey (1929) applied this method to the measurement of the influence of added dry skim milk on the absorption of doughs.

Skovholt and Bailey (1932) found that the farinograph recorded the force applied to the blades of a dough mixer, and used this instrument in studying the increase in absorption effected by dry skim milk. Near and Sullivan (1935) observed that dough consistency as measured with the farinograph was highly correlated with the baker's absorption in a series of flours ranging from 9.3 to 15.6% protein.

Experimental

For the purpose of determining the effects of varying the flour-water ratio five flours were selected, a strong spring wheat straight (No. 16245), a spring wheat clear (No. 15474), a Minnesota winter wheat straight (No. 16246), a family blend patent (No. 16049), and a

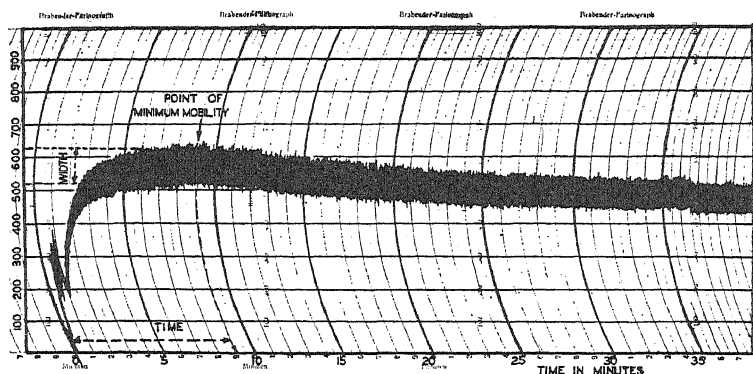


Figure 1. A typical farinograph curve drawn by the pen of the instrument during the mixing of a spring wheat flour dough, exhibiting the methods of measurement of the factors used in this paper.

soft cake patent (No. 15964). Doughs prepared with these flours were mixed in the farinograph with varying amounts of water. The curves drawn by the pen of the instrument were measured for the magnitude of the power-input at the time of minimum mobility, and for the time required to bring the doughs to this point of minimum mobility. The width of the line at the point of minimum mobility was also measured. The method of making these measurements is shown in Figure 1 which represents a typical curve drawn during the mixing of a strong spring wheat flour. The data secured from these flours are presented in Table I.

If the farinograph really measures the viscosity, or its reciprocal the mobility, of doughs then the viscosity with different ratios of flour to water should follow the rule of Sharp and Gortner (1923) for flour suspensions in water. By this rule there should be a curvilinear relation between the water-flour ratio and the viscosity or mobility.

TABLE I

PLASTIC PROPERTIES OF FLOUR-WATER DOUGHS MIXED IN THE
BRABENDER FARINOGRAPH

Flour No. 16246, Minnesota Winter Wheat Straight, 12.00% Protein

Absorption	Point of minimum mobility, B. units	Time to point of minimum mobility	Width of line at point of minimum mobility, B. units	Concentration of flour per 100 grams water	Log. conc. flour	Log. point of minimum mobility	Log. time to point of minimum mobility
%		Min.		G.			
51.5	880	3.0	210	194	2.288	2.945	.477
52	825	3.5	140	192	2.283	2.916	.544
53	750	4.2	140	189	2.276	2.875	.623
54	700	4.2	115	185	2.267	2.845	.623
55	695	7.0	115	182	2.260	2.842	.845
56	595	5.5	110	179	2.253	2.775	.740
57	575	7.5	95	175	2.243	2.760	.875
58	515	7.0	95	172	2.236	2.712	.845
59	490	7.5	85	169	2.228	2.690	.875
60	490	7.7	85	167	2.223	2.690	.887
62	445	9.0	85	162	2.210	2.648	.954
64	390	15.5	50	156	2.193	2.591	1.190
68	325	15.0	60	147	2.167	2.512	1.176
75	250	30.0	40	133	2.124	2.398	1.477

Flour No. 16245, Spring Wheat Straight, 14.3% Protein

60	810	5.5	145	167	2.223	2.909	.740
61	740	6.0	130	164	2.215	2.869	.778
62	735	6.0	125	161	2.207	2.866	.778
63	735	8.0	110	159	2.201	2.866	.903
64	665	6.0	110	156	2.193	2.823	.699
65	655	7.0	100	154	2.188	2.816	.845
67	530	11.0	80	149	2.173	2.724	.964
69	515	13.5	80	145	2.161	2.712	1.130
71	490	14.2	75	141	2.149	2.690	1.152
73	410	22.5	60	137	2.137	2.613	1.352
75	430	28.5	60	133	2.124	2.634	1.455
85	300	41.0	45	118	2.072	2.477	1.613

Flour No. 15474, Spring Wheat Clear, 13.5% Protein

59	855	4.5	175	170	2.230	2.932	.653
60	775	6.5	160	167	2.223	2.889	.813
61	750	7.0	160	164	2.215	2.875	.845
62	730	7.7	155	161	2.207	2.863	.887
64	615	10.7	110	156	2.193	2.788	1.029
66	565	13.7	110	151	2.179	2.752	1.137
68	510	12.7	90	147	2.167	2.708	1.104
70	485	17.2	85	143	2.155	2.686	1.236
72	440	20.7	75	139	2.143	2.644	1.316
74	440	21.7	75	135	2.130	2.644	1.336
78	385	29.5	75	128	2.107	2.586	1.470

TABLE I—*Continued*

Absorption	Point of minimum mobility, B. units	Time to point of minimum mobility	Width of line at point of minimum mobility, B. units	Concentration of flour per 100 grams water	Log. conc. flour	Log. point of minimum mobility	Log. time to point of minimum mobility
<i>Flour No. 16049, Family Patent, 11.0% Protein</i>							
%		<i>Min.</i>		<i>G.</i>			
54	870	2.5	190	185	2.267	2.940	.398
55	775	5.0	135	182	2.260	2.889	.699
56	700	5.0	130	178	2.250	2.845	.699
58	635	6.2	100	172	2.236	2.802	.792
60	555	6.2	85	167	2.223	2.744	.792
60	560	7.0	85	167	2.223	2.748	.845
62	555	7.2	90	161	2.207	2.744	.857
62	510	14.2	70	161	2.207	2.708	1.152
64	445	8.2	65	156	2.193	2.648	.914
66	440	13.7	65	151	2.179	2.644	1.137
68	380	15.5	55	147	2.167	2.580	1.190
70	385	13.2	55	143	2.155	2.586	1.121
74	310	20.0	55	135	2.130	2.491	1.301
80	270	27.0	40	125	2.097	2.431	1.431
<i>Flour No. 15964, Soft Cake Patent, 7.9% Protein</i>							
48	930	1.2	200	208	2.318	2.969	.079
52	580	1.7	100	192	2.283	2.763	.230
55	450	2.5	65	182	2.260	2.653	.398
58	375	4.5	50	172	2.236	2.574	.653
62	300	7.5	35	161	2.207	2.477	.875
67	225	9.5	30	149	2.173	2.352	.978

In Figure 2 this relation for four of the five flours is graphically recorded. When the difference in manner of expression of the data is taken into consideration it can readily be seen that these curves are essentially similar to those of Sharp and Gortner. Sharp and Gortner working on flour suspensions further found that if the logarithm of the flour concentration was plotted against the logarithm of the viscosity the result was a straight line, having a slope which was a function of the properties of the individual flours.

Our data were treated in like manner, with the results recorded in Figure 3, and it is evident that there is a close approximation to the fitted straight lines. In general it appears that the farinograph measures the viscosity or mobility of such doughs fairly well, at least within the range of viscosity here represented.

The relation between the absorption and the time required to bring the doughs to the point of minimum mobility is presented in

Figure 4. This relation is definitely curvilinear, but may be straightened by plotting the logarithm of the time required to reach the point of minimum mobility against the flour concentration in the dough expressed as grams of flour per 100 g. of water, as has been done in Figure 5. These data are rather surprising in that there appears to be

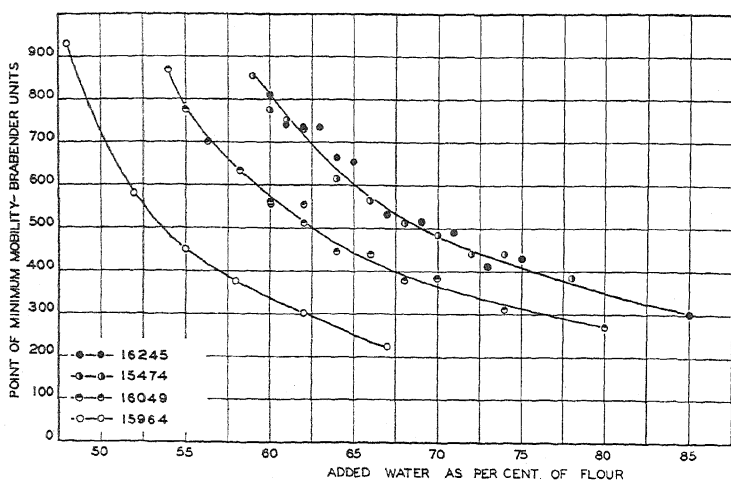


Figure 2. Relation between the amount of water used to form the dough, expressed as percent of the flour, and the minimum mobility of the dough for four typical flours.

no differentiation between the flours, all of which, high-protein spring clear or low-protein cake patent, fall quite well into the linear agreement. This indicates that a high correlation exists between the time required to develop a dough and the amount of water used, even though the mobility at the time of greatest development may vary through wide limits. This relation should receive consideration in developing experimental baking techniques, as it suggests the necessity of departing from a fixed mixing technique with variable amounts of water as specified in the method of the American Association of Cereal Chemists.

The width of the line drawn by the instrument at constant dashpot setting was once suggested as a measure of the elasticity of the dough. The starch-water curves presented in the preceding section of this study (Markley 1937) display quite broad lines, yet it would be absurd to assign any appreciable amount of either tensile or shearing elasticity to a starch-water paste; such a paste may have volume elasticity, but the farinograph is not designed for the measurement of volume elasticity. In Figure 6 is shown the relation between the width of the line and the mobility of the dough at the points of mini-

num mobility. There is no differentiation of the flours and the relation is definitely curvilinear. It would appear from this that the width of the line drawn by the farinograph pen is essentially a function of the mobility of the dough. It is possible from a theoretical aspect that rigidity may enter into this property of width of line in addition to

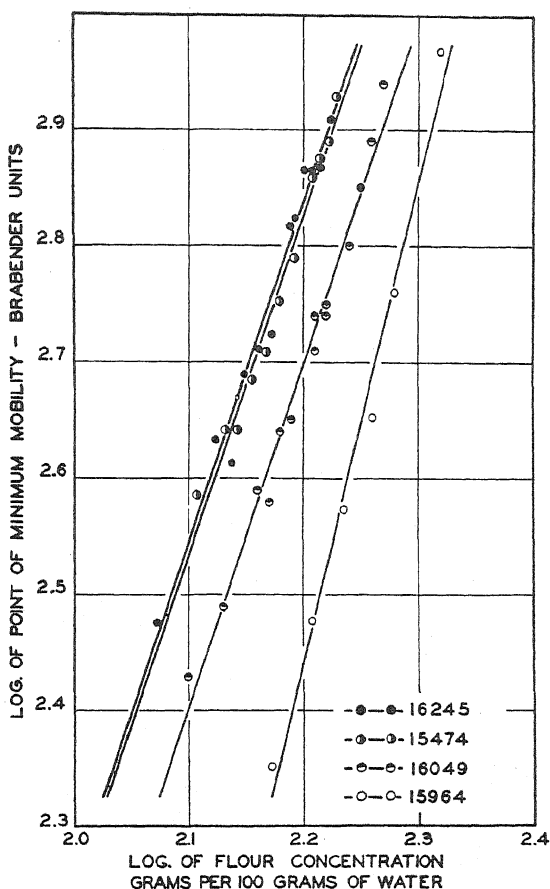


Figure 3. Relation between the flour concentration in the dough, expressed as logarithms of grams of flour per 100 grams of water, and the logarithm of the minimum mobility of the dough in Brabender units for 4 typical flours.

mobility, but as yet we have no precise methods for the measurement of rigidity in doughs. It is quite certain that the width of the line is not a measure of elasticity.

A certain amount of judgment has to be exercised in translating into baking practice the absorption values as determined by the farinograph. Certain flours, as has been shown by Near and Sullivan

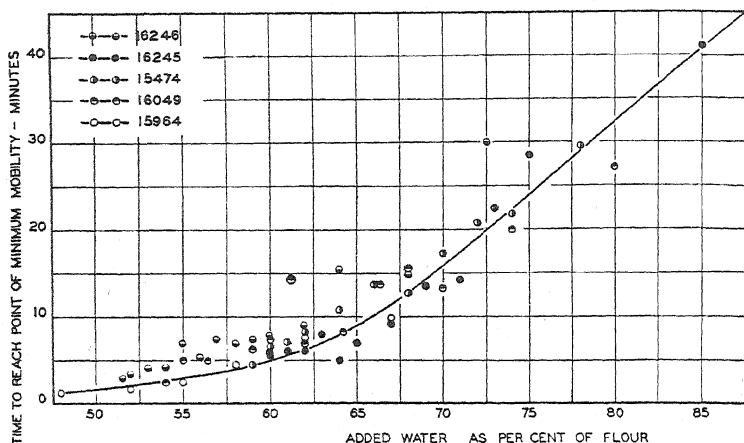


Figure 4. Relation between the amount of water used in forming the dough and the time required to bring the dough to the condition of minimum mobility for 5 typical flours.

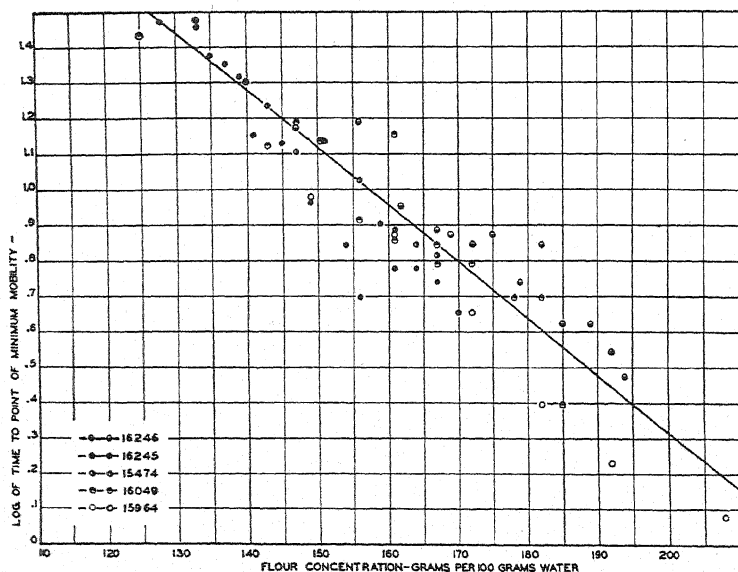


Figure 5. Relation between the flour concentration in the dough, expressed as grams of flour per 100 grams of water, and the logarithm of time required to bring the dough to its condition of minimum mobility for 5 typical flours.

(1935), which tend to slack-off during fermentation must be mixed with less water than the recording dough mixer indicates. This is not a fault of the instrument, but merely means that even though a dough is correct in plastic properties upon leaving the mixer, it may not be in an equally good condition at the conclusion of the fermentation period.

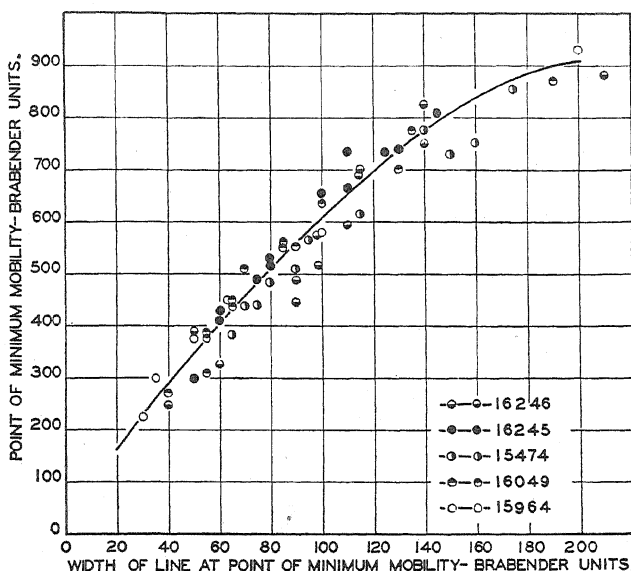


Figure 6. Relation between the width of the line drawn by the pen of the farinograph with the mobility readings in Brabender units at the point of minimum mobility for 5 typical flours.

Summary

Water required to bring doughs to definite minimum mobility can be measured by a recording dough mixer.

Absorption and viscosity, or mobility, in doughs are similar to flour concentration and viscosity in the more dilute flour-water systems studied by Sharp and Gortner with viscosimetric technique.

Absorption and time required to mix a dough are closely related in a curvilinear fashion regardless of the protein content of the individual flours. This line can be straightened by an appropriate mathematical process.

Width of the line drawn by the farinograph pen is a function of the mobility of the dough and not of its elasticity.

Acknowledgments

The authors express their appreciation of the assistance of Frank Harrington in the operation of the farinograph and the aid of Wilfred Dugas in making the many measurements upon the charts used in this and succeeding papers of this series. We gratefully acknowledge grants of funds for labor from the C.W.A., E.R.A., and F.S.A. which enabled this project to be conducted.

Literature Cited

- Bailey, C. H.
1930 A method for measuring the relative plasticity of pastes and doughs. *J. Rheol.* 1: 429-432.
- and LeVesconte, A. M.
1924 Physical tests of flour quality with the Chopin extensimeter. *Cereal Chem.* 1: 38-63 (note particularly p. 49, line 24).
- Borasio and de Rege
1934 Das Pneumodynamometer, ein Apparat zur Qualitätsbestimmung der Mehle. *Das Mühlenlab.* 4: 78-82.
- Briggs, C. H.
1918 Briggs Perfekdo Viscometer. *N. W. Miller*, Jan. 23, p. 275.
- Chopin, M.
1921 Relations entre les propriétés mécaniques des pâtes de farines et la panification. *Bul. soc. encour. ind. nat.* 133: 261-273.
- Fifield, C. C.
1933 A mechanical method for the determination of absorption in bread doughs. *Cereal Chem.* 10: 547-554.
- Halton, P., and Scott Blair, G. W.
1936 A study of some physical properties of flour doughs in relation to their bread-making qualities. *J. Phys. Chem.* 40: 567-569.
- Harrel, C. G.
1927 A gravimetric penetrometer for measuring stiffness or toughness of doughs. *Cereal Chem.* 4: 283-290.
- Jago, Wm., and Jago, Wm. C.
1911 The technology of bread-making. London (note p. 702).
- Kosutany, Thos.
1907 Der ungarische Weizen und das ungarische Mehl. Budapest (for description of Rejto machine, see p. 248).
- Kozmin, N. P.
1936 Das Problem der Backfähigkeit. Leipzig. (For description of Buhler Comparator, see p. 55.)
- Lee, G. van der
1929 Viskosität der Mehlsuspension und Konsistenz der Teiges. *Proc. 1st Internat. Conf. Flour and Bread Mfg. in Prague*, pp. 333-343.
- Markley, Max C.
1937 The colloidal behavior of flour doughs. I. The thixotropic nature of starch-water systems. *Cereal Chem.* 14: 434-436.
- Near, Cleo, and Sullivan, B.
1935 The use of the farinograph as an accurate measure of absorption. *Cereal Chem.* 12: 527-531.
- Skovholt, Oscar, and Bailey, C. H.
1932 The effect of temperature and of the inclusion of dry skim-milk upon the properties of doughs as measured with the farinograph. *Cereal Chem.* 9: 523-530.
- St. John, J. L., and Bailey, C. H.
1929 The effect of dry skim milk upon the water absorption of doughs and the plasticity of flour suspensions. *Cereal Chem.* 6: 140-150.
- Sharp, P. F., and Gortner, R. A.
1923 Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength. *Minn. Agr. Expt. Sta., Tech. Bull.* 19.
- Swanson, C. O., and Working, E. B.
1933 Testing the quality of flour by the recording dough mixer. *Cereal Chem.* 10: 1-29.
- Working, E. B.
1934 The determination of the water absorbing capacity of flour by means of the super-centrifuge. *Cereal Chem.* 11: 527-8.

THE EFFECT OF THE SUBSTRATE ON DIASTATIC ACTIVITY

SUTTON REDFERN and WILLIAM R. JOHNSTON

The Fleischmann Laboratories, Standard Brands Inc.,
New York, New York

(Read at the Annual Meeting, May 1937)

Introduction

Not much attention has been paid to the variability of the soluble starch used for diastatic power determinations; and it is not sufficiently recognized that soluble starches prepared by different workers or manufacturers give different results when used with the same amylase preparations. Lampé (1922) found a difference of 11 to 16% in the diastatic power of a given malt using the soluble starches of Kahlbaum, Merck, Riedel, and Schering. He used the Windisch-Kolbach method and his results were also checked by Windisch. Silbernagel (1930) also showed that the Lintner value of a malt sample depended on the particular lot of soluble starch used in the determination. He suggested the use of starch paste instead of soluble starch, but this is not generally suitable. Recently, Kauert (1937) found that the soluble starch exerted the greatest influence in the determination of diastatic power.

The malt analysis committee of the American Society of Brewing Chemists (1935) in drawing up specifications for the method of determining diastatic power probably also recognized this same difficulty because they laid down very careful specifications for the starch to be used. However, the effect of deviations from these specifications is not stated, and therefore a sample of starch might be rejected and yet satisfy a more fundamental test of its suitability as given later.

Snider and Coleman (1937) have discussed the suitability of starch as a substrate for amylase in terms of its reducing power, pH, erythro-dextrin content, and Lintner value of a standard malt. They concluded, among other things, that a low phosphate (or low ash) content is desirable for optimum diastatic activity; that the reducing substances in soluble starch should not exceed 1% in terms of maltose; and that soluble starch solutions before addition of buffer should have a pH of 4.6.

Sallans and Anderson (1937) found that the standard error for starches is of the order of $\pm 3\%$.

It became necessary while developing a new method for determining diastatic power by application of the principles given by Johnston and Jozsa (1935) to set up a standard for soluble starch which could be duplicated without comparison with a previous batch of starch. The most descriptive method of studying the influence of the soluble starch on diastatic action is by a study of the kinetics of the reaction. Hanes (1932) has already pointed out the advantages of this method. In this manner by maintaining all variables—pH, temperature, quantity of enzyme, starch, concentration of substrate—constant except the starch, the effect of the starch is revealed. The rate curve method of study demands an extended experimental procedure, but it gives the most complete and reliable information of any method.

Experimental

REAGENTS

The ferricyanide method of sugar analysis is probably the most convenient method for estimating the reducing substances formed by diastasis and was therefore chosen for this work. The following reagents are required:

Standard sodium thiosulfate solution: Dissolve 12.41 g. of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in distilled water which has been previously boiled and cooled and dilute to one liter. Standardize this solution against the 0.1 *N* KIO_3 solution by pipetting 12.50 ml. of the KIO_3 solution into an Erlenmeyer flask, adding 5 ml. of 50% KI and 4 ml. of 6 *N* HCl (in the order named). Titrate immediately with the thiosulfate using 3 ml. of starch indicator toward the end of the titration. The solution is preserved by the addition of a few drops of CS_2 .

Standard potassium iodate solution: Dissolve 3.5672 g. of KIO_3 in distilled water and dilute to one liter. The concentration of this solution is exactly 0.1 *N*.

Alkaline ferricyanide solution: Dissolve 16.5 g. of C.P. $\text{K}_3\text{Fe}(\text{CN})_6$ and 22 g. of Na_2CO_3 in distilled water and dilute to one liter. This solution must be protected from light. Standardize it against the thiosulfate solution by pipetting 25.00 ml. of the ferricyanide solution into a 300 ml. Erlenmeyer flask, adding 25 ml. of the ZnSO_4 reagent, then 5 ml. of 50% KI, and titrating immediately with the thiosulfate. If the solution is not exactly 0.05 *N*, adjust it to this strength.

Zinc sulfate reagent: Dissolve 50 g. of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 100 g. of KCl in 600 ml. of distilled water. Add 200 ml. of glacial acetic acid and dilute to one liter. Crystals of $\text{K}_2\text{SO}_4 \cdot \text{ZnSO}_4 \cdot 6\text{H}_2\text{O}$ will gradually settle out on standing, but this will not harm the solution.

Starch indicator solution: Make a 1% solution of soluble starch in water. Preserve by the addition of a few drops of toluene, which is just as satisfactory as NaCl and more convenient to use.

Acetate buffer solution: To 1.10 mols of acetic acid is added sufficient approximately 4 *N* carbonate-free NaOH solution so that when the mixture is diluted to one liter the pH will be 5.00. About 0.73 mols of NaOH will be necessary. The total acetate concentration of this buffer solution is 1.10 molar and is so calculated that when 50 ml. of it are used for each liter of starch solution and 100 ml. of starch solution are mixed with 10 ml. of malt solution, the final buffer concentration will be 0.05 molar. This concentration is sufficient to insure maximum activation of the amylase according to the work of Sherman, Caldwell, and Boynton (1930).

2 N NaOH and HCl solutions: It is not necessary that these solutions be exactly 2 *N*, but they should be equivalent, *i.e.*, 10 ml. of the NaOH should just neutralize 10 ml. of the HCl.

Starch solution: Mix 20 g., based on dry weight, of soluble starch with a small quantity of distilled water and pour while stirring into 750 ml. of boiling distilled water. Continue boiling for 2 to 3 minutes after the starch is added. Then cool the starch solution and rinse into a one-liter volumetric flask. Add 50 ml. of acetate buffer and dilute to volume. The water content of the starch is readily determined by drying 5 g. of it for one hour at 120° C. in a forced-draft drying oven.

Malt solution: Exactly 5 g. of a highly active (approximately 261° L.) malt syrup are weighed out in an aluminum weighing scoop and transferred to a 500 ml. volumetric flask and made to volume with distilled water. The malt syrup is kept in a glass-stoppered bottle and stored in the ice box at 3.3° C. No loss in activity occurred during the time of these experiments. The malt syrup is used in preference to dry malt because of the convenience in making up a fresh solution each day.

Procedure for Determining Rate Curves

Hanes (1932) described a very satisfactory procedure for studying the early stages of the saccharification of soluble starch by malt amylase. The following procedure, based on Hanes, is used to determine the rate curves.

The reaction is carried out in 300 ml. Erlenmeyer flasks supported in a large water thermostat maintained at $25.00 \pm 0.05^\circ$ C. All pipets used are protected with cotton plugs to exclude traces of saliva. United States Bureau of Standards calibrated pipets are always used except for adding the starch solution.

Before a run is started, the solutions to be used are allowed to come to the temperature of the water bath; and 50 ml. portions of the alkaline ferricyanide solution are pipetted into 300 ml. Erlenmeyer flasks from an automatic pipet.

Ten milliliters of the malt solution are pipetted into an Erlenmeyer flask. After waiting a few minutes to insure temperature equilibrium, 100 ml. of the starch solution are added from a fast (30-second) pipet noting the time with a stopwatch. About one minute before the time the first sample is to be taken, a clean, dry pipet is filled with the reaction mixture and allowed to drain back into the flask. Then 30 seconds before the desired time, the pipet is refilled and the level adjusted to the mark. The tip is then freed from adhering drops and inserted into one of the flasks containing alkaline ferricyanide. At the desired time—2, 4, 6, etc., minutes—the sample is delivered into the ferricyanide. The delivery is hastened by blowing out most of the liquid, allowing, however, the last drops to drain out in the time specified on the pipet, while holding the tip of the pipet against the side of the flask. The flask is then gently rotated to wash down the sides. The procedure for subsequent samples is the same. A 10 ml. pipet is used for the 2, 4, 6, 8 and 10-minute samples, and a 5 ml. pipet for the 15, 20, and 30-minute samples.

Hanes (1932) showed that his alkaline ferricyanide reagent will kill the amylase. By direct measurement, the pH of the alkaline ferricyanide used here is found to be approximately 12. Ohlsson (1930) found that at 38° C. and pH > 10.5 both alpha- and beta-amylase are completely killed, which shows that the alkalinity of the ferricyanide is sufficient to arrest diastatic action completely.

The blank is determined by pipetting 100 ml. of starch solution into a 200 ml. volumetric flask, adding 10 ml. of 2 *N* NaOH, 10 ml. of malt solution, and 10 ml. of 2 *N* HCl, in the order named. The solution is made to volume, and a 25 ml. aliquot is taken for analysis. The initial reducing power is determined on a 25 ml. sample of the original starch solution. The same result is obtained with or without buffer salts present.

Determination of Reducing Substances

The reducing substances formed in the starch, in the blank mixture, and those formed by the amylase are then estimated by a slight alteration of Gore and Steele's (1935) modification of the Blish and Sandstedt (1933) ferricyanide method.

Sufficient distilled water is added to the ferricyanide-sugar solution to make a total volume of 75 ml. The flasks are then heated in a boiling water bath for exactly 20 minutes and quickly placed in the

thermostat at 25°. After the flasks have cooled for at least five minutes and not more than one hour, 50 ml. of the ZnSO_4 reagent and 5 ml. of the KI solution are added and the solution immediately titrated with the standard thiosulfate.

The flasks are supported during the boiling on a false bottom made of $\frac{3}{8}$ -inch mesh galvanized wire screen which insures uniform heating. Since the oxidation of sugars by ferricyanide is an incomplete reaction, all determinations must be carried out in exactly the same manner. Uniform cooling of the flasks in a thermostat gives more consistent results than cooling in tap water. In this laboratory tap water varies from 7° in the winter to 21° C. in the summer. The oxidized sugar solutions must not be allowed to stand after adding the ZnSO_4 reagent because, as shown by Putnam, Blish, and Sandstedt (1935), an increase in maltose values occurs.

The pH of the 2% starch solutions, without buffer, is measured with a Hellige glass electrode calibrated against 0.05 molar acid potassium phthalate. The amount of erythrodextrin is estimated qualitatively by Snider and Coleman's (1937) modification of Small's (1919) method.

Maltose Standardization

The relation of the milliliters of 0.05 *N* ferricyanide consumed to maltose oxidized was determined against pure recrystallized maltose hydrate. The specific rotation of the sample used was $[\alpha]_D^{22.6^\circ} = +130.8^\circ$. Varying quantities of this maltose were placed into 300 ml. Erlenmeyer flasks, 50 ml. of ferricyanide added and the oxidation carried out as previously described. From these results it is found that the relation between milliliters of ferricyanide and milligrams of maltose hydrate is linear as far as 84 mg. From these data the equation of the best straight line was derived by the method of least squares and is $M = 1.791 F$ ($M < 84$ mg.),

where M = number of milligrams of maltose hydrate

and F = number of milliliters of 0.05 *N* ferricyanide.

Using this relation it is easy to calculate the amount of maltose formed in each interval of time. All results are corrected by subtraction of the blank, part of which is due to the malt syrup and part to the starch.

Soluble Starches Used in the Study

Thirteen soluble starches were used. Ten of them were of commercial grade and three were prepared in the laboratory. Two of the laboratory preparations were prepared, after Lintner (1908), by

covering BKMF potato starch with 7.5% HCl, and allowing it to stand for 7 days at approximately 18° C. The starch was then filtered off, washed with distilled water, the remaining acid neutralized with NaHCO₃ (using neutral "lacmus" paper as indicator), and finally washed free from chloride. It was then air dried and ground. The other starch was prepared by Gore's (1928) method. It will be noticed that all of these starches are not Lintner starches. The other starches were included in order to compare them with the Lintner starches which have been accepted generally as a standard in the study of diastatic power. Soluble starches prepared by the Lintner method are not especially unique, but the method is easy, inexpensive, not too drastic, and gives a starch relatively free from impurities. A description of these starches will be found in Table I.

TABLE I
DESCRIPTION OF THE STARCHES

Soluble starch number	Nature	Origin	Labeled
1	Laboratory preparation	Potato	Lintner
2	"	"	"
3	Commercial	"	"
4	"	"	"
5	"	"	"
6	"	"	"
7	"	"	Improved Lintner
8	"	"	
9	"	"	Indicator Practical C.P. C.P.
10	"	"	
11	"	"	
12	"	"	
13	Laboratory preparation	"	

Discussion of Rate Curves

A rate curve was run for each of these starches. Each curve is the average of two experiments. The average deviation from the mean is not more than 2% and in most cases is about 1%. This seems to be the precision attainable by this method. Figure 1 shows the rate curves for four of these starches which are characteristic of all. It can be seen that all of these curves are linear for approximately the first ten minutes, and the rate at zero time is easily determined from the large-scale graph on which these curves were constructed. The data from which these curves were constructed as well as those for the remainder of the rate curves given in this paper are conveniently assembled in Table II.

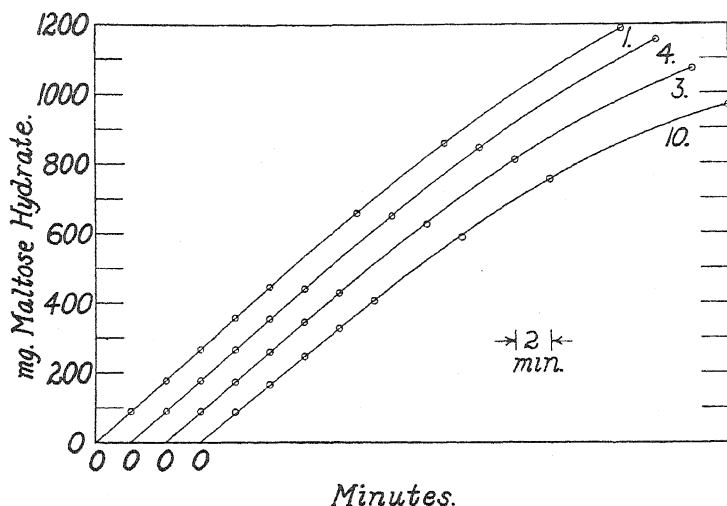


Figure 1

TABLE II
DATA FOR ALL RATE CURVES

Time, minutes Starch number	Mg. of maltose hydrate (average values)							
	2	4	6	8	10	15	20	30
1	89	177	267	357	446	658	859	1189
2	91	178	266	354	441	647	849	1182
3	88	171	258	343	427	626	810	1071
4	89	177	265	353	440	649	846	1156
5	92	179	269	357	445	650	852	1170
6	91	174	264	352	441	644	843	1161
7	90	178	268	355	437	645	837	1121
8	90	178	265	354	439	642	837	1119
9	86	168	250	332	412	601	775	1015
10	86	166	246	327	406	589	757	965
11	92	179	270	360	450	660	863	1182
12	89	178	267	356	444	648	849	1179
13	93	181	273	362	453	672	887	1236
3F	92	178	266	355	439	640	832	1109
4F	89	176	265	354	439	648	849	1170
9F	88	174	259	344	426	627	817	1097
10F	87	171	254	337	418	613	790	1036
50% 4F	92	177	265	352	435	636	828	1116
50% 10F								
25% 4F	87	175	258	344	424	618	802	1069
75% 10F								

Table III gives the moisture content, initial rate, reducing power, and maltose formed in 30 minutes. By giving the amount of maltose formed in 30 minutes along with the initial rate, the curve is defined more completely.

TABLE III
CHARACTERISTICS OF THE SOLUBLE STARCHES
(Arranged in order of increasing initial rate)

Starch number	Initial rate, Mg. maltose ¹ per minute	R.P. % maltose ¹	pH	Erythro-dextrin	Mg. maltose ¹ in 30 min.	Moisture %
10	41.1	11.31	3.50	++++	965	10.37
9	41.7	11.74	3.40	++++	1015	12.09
3	42.9	5.47	3.55	++++	1078	18.22
6	44.2	1.96	5.05	+	1161	13.31
8	44.2	2.65	5.60	++++	1119	17.38
4	44.3	3.06	3.83	++	1156	11.04
7	44.3	4.15	4.20	+++	1121	8.04
12	44.4	1.26	6.00	++	1179	14.64
2	44.5	1.57	5.85	+	1182	12.88
1	44.6	1.93	6.45	+	1189	7.64
5	44.8	1.97	4.96	+	1170	13.77
11	44.9	3.33	5.90	+++	1182	12.18
13	45.4	10.05		++++	1236	9.38

¹ Maltose in this paper actually means maltose hydrate.

A study of Figure 1 and Table III reveals several facts. First, we can note the approximate exactitude of Kjeldahl's law if we use it in its modern form which is given by Ling (1936) as follows: "When malt extract is allowed to act on starch paste—at the present time soluble potato starch solution is used—and the saccharification measured by the reducing power of the conversion liquid, expressed in terms of maltose, the saccharifying power is a linear function of the time or mass, provided the reducing power expressed as percent of maltose on the dry starch does not exceed 30%." In the present case this amounts to 630 mg. of maltose hydrate. The rate curves in all cases are only strictly linear for ten minutes or to approximately 450 mg. of maltose; the deviation from linearity at 630 mg. is small, but nevertheless distinct.

A second important conclusion is that there is no correlation between reducing power and initial rate. This is contrary to what had been expected in view of the importance attached to it by the American Society of Brewing Chemists. As can be seen, values of R.P. from 1.26% to 4.15% give practically the same initial rate. A low initial R.P., however, is desirable because, as both Clark (1912) and Hanes (1935) have pointed out, we cannot correct exactly for the initial R.P. without information concerning the nature of the reducing substances and their fate during diastasis. The authors feel, nevertheless, that since it is impossible to get a soluble starch without any initial R.P.—even raw starch paste has 0.15% R.P.—more uniform results are obtained if the blank is used. Our results indicate that a blank correction is necessary in order to attain a precision of $\pm 2\%$.

As is easily seen there is also no relation between the pH of the unbuffered starch solution and the initial rate. Variations from pH 3.83 to 6.45 show practically the same initial rate. This result is surely to be expected. However, Snider and Coleman attempted to show that "the problem with respect to the acidity of soluble starch . . . is not, however, its adjustment to a pH of 4.5 to 4.7 by means of buffers, but rather which starch is more desirable—the one close to the starch neutral point ($\text{pH} = 4.6$) on the acid side, or the one close to the starch neutral point on the alkaline side." The activity of malt amylase is determined with respect to pH only by the pH of the substrate solution when buffered and not upon its previous pH history.

Snider and Coleman also concluded that a low phosphate content, indicated by a low ash content, of soluble starch is desirable for optimum diastatic activity. The authors could not conclude this from Snider and Coleman's data, and furthermore do not consider it of importance when acetates are present in a large quantity as they are in the Lintner determination. Sherman, Caldwell, and Boynton (1930) showed that malt amylase exhibited the same activity in starch solutions containing the same concentration, 0.01 *M*, of acetate and phosphate provided the systems had been adjusted to their respective pH-optima. The pH-optima for acetate and phosphate differ by approximately 0.1 to 0.2 pH units and in the case of the Lintner determination must be conditioned only by the large excess of acetate ion present.

The relative quantity of erythrodextrin does not seem to affect the initial rate as can be easily seen, and therefore is not as critical a factor as suggested by Snider and Coleman.

Certain aspects of the data of Table III demand further consideration. Starch No. 3 is a Lintner starch and yet it shows a lower initial rate than the rest of the Lintner starches. Starch No. 13 has a high R.P. and despite this has the highest initial rate of any starch studied, quite in contrast with starches Nos. 9 and 10 which have about the same R.P. but very low initial rates. Lüers and Wasmund (1922) found that maltose inhibits diastasis, and Ohlsson (1930) found that dextrins are saccharified more easily than either soluble starch or starch paste. These two papers suggested that perhaps starch No. 13 consists largely of dextrins and very little reducing sugars, whereas starches Nos. 3, 9, and 10 contained more reducing sugars which tend to slow down the reaction.

Determination of Fermentable Sugars

In order to determine the quantity of reducing substances present, whether maltose, glucose, or perhaps low dextrans, the biological method of Schultz and Kirby (1933) was selected as suitable. No attempt was made to differentiate different sugars, but all substances fermentable by baker's yeast are calculated as maltose. The method of analysis slightly modified from Schultz and Kirby is as follows. The apparatus used is exactly the same. One hundred milliliters of water, 1.5 g. of $\text{NH}_4\text{H}_2\text{PO}_4$, 0.6 g. of $(\text{NH}_4)_2\text{HPO}_4$, 1 g. of maltose, and 3 g. of fresh baker's yeast are shaken together until the initial charge of maltose is completely fermented. The shaking is then stopped, a weighed sample (10 to 20 g.) of the starch is added and shaking resumed until fermentation is complete. By assuming 210 ml. of CO_2 to be equivalent to 1 g. of maltose, the percent of fermentable substances calculated as maltose is easily obtained. The large gasometers which were available make the results only approximate, but what is more important the relative magnitude and order are correct. The results are given in Table IV in increasing order of percent fermentable substances and repeating the initial rate and R.P. for comparison.

TABLE IV
QUANTITY OF FERMENTABLE SUBSTANCES IN SOLUBLE STARCHES

Starch number	Fermentable substances as % maltose	R.P. % maltose	Initial rate, Mg. maltose per minute
1	0.34	1.93	44.6
11	0.37	3.33	44.9
4	0.41	3.06	44.3
2	0.42	1.57	44.5
8	0.44	2.65	44.2
7	0.49	4.15	44.3
6	0.58	1.96	44.2
5	0.74	1.97	44.8
13	1.0	10.05	45.4
3	1.8	5.47	42.9
9	3.0	11.74	41.7
10	3.6	11.31	41.1

It can be seen that there is a good agreement between the amount of fermentable substances and the initial rate. Of course it is realized that the initial rate is influenced by more factors than just this one, but in general when the percent of fermentable substances is low, the initial rate is what we may call normal for soluble starches and averages 44.5 mg. of maltose per minute for the standard quantity of malt syrup used in testing. The difference between starches Nos. 3 and

13 is quite apparent. Starch No. 3 has a lower rate than No. 13 although the R.P. of No. 13 is almost twice that of No. 3. Nos. 9 and 10 with a large percentage of fermentable substances have the lowest rates of all. There is no relation at all between fermentable sugars and R.P.

Kinetics of Fermented Starches

It was now thought that perhaps here is the suggestion for a method of making the rate curves for all these starches coincide if they are equally susceptible to diastase. Several of these starches were then fermented with yeast in the following manner to remove all fermentable substances. Three grams of $\text{NH}_4\text{H}_2\text{PO}_4$, 1.2 g. of $(\text{NH}_4)_2\text{HPO}_4$, 3 g. of fresh baker's yeast, 150 g. of soluble starch, and 400 ml. of distilled water are mixed together in a one-liter Erlenmeyer flask. The flask is placed in a shaking apparatus and shaken overnight which insures complete fermentation. The starch-yeast mixture is now filtered on a Büchner funnel. The mixture is suspended in distilled water and divided into four parts which are poured into 250-ml. centrifuge bottles. The mixture is centrifuged until the starch is well packed down. Most of the yeast comes to the top and is removed by scraping with a spatula. The starch is suspended again in water and separation repeated. A third time suffices to remove the yeast almost completely. The starch is then filtered again on a Büchner funnel, washed, sucked dry, and dried two days between filter paper. After grinding, the moisture content is determined in the usual manner. The yields varied from 60 to 85% depending on the care used in scraping off the yeast. A yeast count made on one sample gave 7000 cells per gram in the fermented starch as against 400 cells per gram in the original starch. This illustrates the success of this simple method of yeast removal. The very small percent, approximately 0.001% by weight, of yeast remaining does not interfere with the sugar determination because it must be remembered that the starch solution is boiled before use which effectively kills all the yeast cells and their concomitant enzymes.

Starches Nos. 3, 4, 9, and 10 were treated by this fermentation method and rate curves were determined for each. Table V gives the R.P., initial rate, and the amount of maltose formed in 30 minutes compared with the same values on the original starch.

In all four starches the residual R.P. is always less than the difference between the original R.P. and quantity of fermentable sugars, showing definitely that the fermentable substances are not all maltose. The R.P. of starches Nos. 9 and 10 is reduced quite remarkably by fermentation. The initial rate of starch No. 3 has been raised from

TABLE V
CHARACTERISTICS OF THE FERMENTED STARCHES

Starch number	3F	4F	9F	10F
Prepared from soluble starch number	3	4	9	10
R.P. % maltose				
Before	5.47	3.06	11.74	11.31
After	3.16	2.31	5.42	5.27
Initial rate, Mg. maltose/min.				
Before	42.9	44.3	41.7	41.1
After	44.6	44.5	43.4	42.4
Mg. maltose in 30 min.				
Before	1078	1156	1015	965
After	1109	1170	1097	1036

42.9 to 44.6 mg. of maltose per minute, which is the normal value, but the rate drops off more rapidly after 10 minutes as shown by the 30-minute value, 1109 mg. The average normal value at 30 minutes is 1174 mg. of maltose. Therefore not all of this starch is apparently equally susceptible to diastase. The initial rates of both Nos. 9 and 10 are increased, but not up to the normal value, indicating that these starches are also not quite as susceptible to diastase as a normal starch. Starch No. 4, which is what may be called a normal starch (defined more completely later), is affected very little by this fermentation treatment.

To help decide whether starch No. 10 is less susceptible towards diastase, the rate curves for two mixtures of fermented starches Nos. 4F and 10F were determined, and the results are given in Table VI, which are compared with the same values for the separate starches.

TABLE VI

Composition of mixture	Initial rate	Maltose in 30 minutes
50% 4F 50% 10F	44.0	1116
25% 4F 75% 10F	43.1	1069
4F	44.5	1170
10F	42.4	1036

It will be noted that the initial rate of the 50-50 mixture is 44.0 mg., whereas the corresponding rate for the 25-75 mixture is 43.1 mg. This can be accounted for on a statistical basis. The enzyme molecules acting on the 50-50 mixture make, since we are dealing with a statistical system, more collisions with susceptible starch molecules than when acting on the 25-75 mixture. Accordingly, the effect of

the proportion of susceptible molecules will be reflected in the initial rate. The 44.0 mg. initial rate of the 50-50 mixture is approximately equal to the corresponding rate for starch 4F, showing that for the first few minutes the enzyme molecules make sufficient collisions with susceptible starch molecules to permit maintenance of rate. However, as the proportion of susceptible molecules diminishes the rate falls off rapidly. This is borne out by the 43.1 mg. initial rate of the 25-75 mixture.

The rate-curve study of these fermented starches leads us to believe that some of these starches have been altered in some manner during the process of manufacture so that they differ in their internal structure and cannot be made to act normally without effecting structural changes. We have no explanation for these variations but are planning further work which may clarify the situation.

Definition of Normal Starch

From the results of all these studies, the soluble starches studied can be separated into two groups as shown in Table VII.

TABLE VII
SACCHARIFICATION INDEX OF SOLUBLE STARCHES

	Starch number	Initial rate A	Maltose in 30 minutes B	Ratio $\frac{B}{A}$
Group A	1	44.6	1189	26.6
	2	44.5	1182	26.6
	4	44.3	1156	26.1
	5	44.8	1170	26.1
	6	44.2	1161	26.3
	11	44.9	1182	26.4
	12	44.4	1179	26.5
Group B	3	42.9	1078	25.2
	7	44.3	1121	25.4
	8	44.2	1119	25.3
	9	41.7	1015	24.4
	10	41.1	965	23.4
	13	45.4	1236	27.2

Those starches in Group A are what we may call normal. By normal we mean that when used with the same amylase concentration all starches in this group will give the same rate curve within a precision of $\pm 2\%$. Or expressed in another way, the same Lintner value for the enzyme preparation will be obtained when using any starch of Group A as the substrate. All starches in Group B will not give the same rate curve; and may or may not give the same Lintner value. Starches Nos. 7 and 8 would give the same Lintner value as the

starches of Group A because they have a normal initial rate and the Lintner method utilizes only the linear portion of the rate curve. However, their 30-minute conversion points are low and therefore their rate curves do not coincide with the normal rate curves of Group A.

In column 4 of Table VII we have listed the calculated ratios of the amount of maltose formed in 30 minutes to the initial rate for all starches studied. This ratio may be identified as an index to the suitability of a given starch for enzyme concentration measurements. From the data given in Table VII and other data not tabulated in this paper, it is found that a normal starch has a saccharification index from 26.1 to 26.8. Consequently we suggest that a normal starch may be chosen from an unknown group of soluble starches by carrying out a saccharification reaction using sufficient enzyme to hydrolyze the starch at an initial rate from 44 to 45 mg. of maltose hydrate per minute. The enzyme used shall be preferably a high diastatic malt syrup. If a dry malt must be used then it shall be extracted with 5% NaCl solution substituted for the water in the official method. The necessity of using NaCl for the extraction has been proved by repeated tests and will be reported in a future paper. If the saccharification index is within the limits 26.1 to 26.8, then the investigator may conclude that he has a normal starch at hand. It may be added that the starch should also have as low an initial reducing power as possible.

Anderson (1937) points out that "the possibility of differences of 10% between laboratories, occurring merely as a result of differences in the starches cannot be viewed with equanimity." He further states that in his opinion the difficulty can not be adequately overcome by adopting more stringent specifications for the starch because the testing of the starch would be too difficult.

The authors feel that the two specifications presented here are simple enough for any routine laboratory to apply and yet exact information is given concerning the suitability of a given starch.

Literature Cited

- American Society of Brewing Chemists
1935 Official Methods of Analysis. Published by A. S. B. C., Chicago.
Anderson, J. Ansel
1937 Diastatic power in malt. *Modern Brewer* 18: No. 5, 57-60.
Blish, M. J., and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* 10: 189-202.
Clark, Ernest D.
1911-12 A study of Lintner soluble starch. *Biochem. Bull.* 1: 194-206.
Gore, H. C.
1928 A new soluble starch and an improved polarimetric Lintner method. *Ind. Eng. Chem.* 20: 865-867.

- Gore, H. C., and Steele, H. K.
1935 Estimation of the saccharifying power of malt diastase. *Ind. Eng. Chem. Anal. Ed.* 7: 324-326.
- Hanes, Charles S.
1932 The effect of starch concentration upon the velocity of hydrolysis by the amylase of germinated barley. *Biochem. J.* 26: 1406-1421.
1935 The action of the two amylases of barley. *Can. J. Research* 13B: 185-208.
- Johnston, W. R., and Jozsa, S.
1935 A general method for determining the concentration of enzyme preparations. *J. Am. Chem. Soc.* 57: 701-706.
- Kauert, G.
1937 *Pet. J. Brasseur* 45: 215, through *International Brewing Abstracts* 605/45.
- Lampé, B.
1922 Einige Beobachtungen über den Einfluss der löslichen Stärken verschiedener Herkunft bei der Bestimmung der diastatischen Kraft im Malz nach Windisch und Kolbach. *Wochschr. Brau.* 39: 31.
- Ling, A. R.
1936 Advances in the knowledge of malt conversion during the last fifty years. *J. Inst. Brewing* 42: 504-509.
- Lüers, H., and Wasmund, W.
1922 Über die Wirkungsweise der amylase. *Fermentforschung* 5: 169-235.
- Ohlsson, E.
1930 Über die beiden Komponenten der Malzdiastase, besonders mit Rücksicht auf die Mutarotation der bei der Hydrolyse der Stärke gebildeten Produkte. *Z. physiol. Chem.* 189: 17-63.
- Putnam, H. W., Blish, M. J., and Sandstedt, R. M.
1935 Factors affecting maltose values in the ferricyanide method for diastatic activity. *Cereal Chem.* 12: 494-504.
- Sallans, Henry R., and Anderson, J. Ansel
1937 Sources of error in the determination of the diastatic power of malt. *Cereal Chem.* 14: 708-720.
- Schultz, A., and Kirby, G. W.
1933 Biological method for the determination of different sugars in starch degradation products. *Cereal Chem.* 10: 149-155.
- Sherman, H. C., Caldwell, M. L., and Boynton, H. H.
1930 A quantitative study of the influence of acetate and of phosphate on the activity of malt amylase. *J. Am. Chem. Soc.* 52: 1669-1672.
- Silbernagel, F. C.
1930 Determination of the relative diastatic powers of malt. *Ind. Eng. Chem. Anal. Ed.* 2: 31-32.
- Small, James Craig
1919 Quantitative determination of soluble starch and its hydrolytic cleavage products. *J. Am. Chem. Soc.* 41: 107-112.
- Snider, S. R., and Coleman, D. A.
1937 Soluble starch: A study of its properties with a recommended supplementary procedure for determining its suitability for use in the Lintner determination. *Cereal Chem.* 14: 1-17.
- Wirth, C., and Lintner, C. J.
1908 Über die Bestimmung der diastatischen Kraft des Malzes und von Malz-extrakten. *Z. ges. Brauw.* 31: 421-425.

THE MEANING OF FERMENTATION TOLERANCE

ROWLAND J. CLARK

Department of Milling Industry, Kansas State College, Manhattan, Kansas

(Read at the Annual Meeting, June 1936)

In considering the subject of fermentation tolerance, information was desired upon what this term meant to cereal workers. A questionnaire was therefore sent out requesting a definition of the term. The author wishes to express his sincere appreciation to the cereal chemists, bakers and bakery engineers named below for their answers to this questionnaire sent them by the writer. The ideas expressed by these workers in their replies have formed the basis for the following discussion on fermentation tolerance.

A. W. Alcock, Winnipeg, Manitoba; Carl L. Alsberg, Stanford University, Calif.; M. J. Blish, Lincoln, Nebr.; Ralph Bohn, Chicago, Ill.; Mary M. Brooke, Omaha, Nebr.; L. E. Caster, Rockford, Ill.; A. E. Curtis, Kansas City, Mo.; Claude F. Davis, Salina, Kans.; R. K. Durham, Kansas City, Mo.; Charles N. Frey, New York, N. Y.; George F. Garnatz, Cincinnati, Ohio; W. F. Geddes, Winnipeg, Manitoba; C. G. Harrel, Minneapolis, Minn.; W. L. Heald, Kansas City, Mo.; L. E. Jackson, Chicago, Ill.; Arnold H. Johnson, Baltimore, Md.; I. O. Juvrud, Chicago, Ill.; R. K. Larmour, Saskatoon, Saskatchewan; L. E. Leatherock, Wichita, Kans.; C. A. McDuffee, Joplin, Mo.; W. J. Ocken, Chicago, Ill.; L. R. Olsen, Minneapolis, Minn.; Peter G. Pirrie, New York, N. Y.; John Roberts, Los Angeles, Calif.; A. A. Schaal, Cambridge, Mass.; B. J. Sullivan, Minneapolis, Minn.; E. F. Tibbling, Kansas City, Mo.; Wm. Walmsley, Chicago, Ill.

There were 28 replies to the questionnaire. Three replies stated that fermentation tolerance of a dough was dependent entirely upon the sugar level or gassing power of the dough; two replies were so general and indefinite that no specific definition was given; while 23 replies expressed very closely the ideas embodied in the following paragraphs.

Three of the 23 replies defined fermentation tolerance so concisely that they are quoted at length as a theme for more detailed consideration.

Reply No. 1. "I regard fermentation tolerance as the length of time over which a good loaf of bread (judged from the standpoint of flavor, volume, texture, etc.) can be produced from a given flour dough. The proper balance between gas production and gas retention (the latter factor being dependent on the 'quality' and quantity of gluten) is the most important factor governing the fermentation tolerance of a flour. In my opinion, fermentation tolerance is dependent fully as much on the amount of gluten and its physical characteristics as on the sugar content of the dough."

Reply No. 2. "My understanding of fermentation tolerance is

the range of time during which the two main factors of bread baking are in balance, that is, gas production and gas retention, and produce quality bread."

Reply No. 3. "To me, the fermentation tolerance always meant the range of time over which the gas production and gas retention could be kept in the proper balance to produce satisfactory bread."

All the reactions and procedures involved in bread making, may be divided into three groups, namely: mixing, fermentation and baking. Under mixing would be found the changes which take place in the dough batch as the various ingredients are brought together and incorporated into a homogeneous mass. Each flour requires a very definite period of time for its optimum mechanical development which varies according to its own varietal characteristics, the temperature of mixing, the kind and proportion of materials used, the speed of the mixer and other conditions imposed. This mixing period is dependent upon or is the resultant of all these forces reacting upon each other simultaneously and the end of the period or the point of optimum dough development is very definitely indicated to the baker by the familiar "clean-up" in the mixer. No stated time can be assigned for a mixing period until this "clean-up" is observed. Following this test a mixing time can be stated provided all the materials and conditions remain constant, a situation which is very difficult to maintain. When the dough "cleans up" in the mixer it indicates that an equilibrium has been reached between all the forces of absorption, ingredients, temperature, mixing speed and other factors. This is the pivotal point of dough development mechanically. The dough should be dumped from the mixer, either before, upon or after the "clean-up" depending upon the flour in question.

Now begins the period of fermentation. It is started with a dough in a balanced condition. If the fermentation period were skipped and the dough baked immediately upon coming from the mixer, the bread would be solid, heavy, hard, flavorless and unpalatable. In order to aerate the dough, make it light and give the bread volume, it is necessary to generate gas within the mass. Two groups of forces now start to operate, namely, those of gas production and of gas retention. Gas in the dough is generated largely by the enzymes diastase, maltase and zymase. The gas retention or colloidal dispersion of the dough is developed by enzymes (principally protease), by chemical means (ash, moisture, H-ion, bleach, oxidizers, etc.) and by some mechanical agencies (stretching under gas formation, punching, dividing, rounding and molding). Therefore, the objective to be accomplished during the fermentation division of the bread making process is to generate sufficient gas for maximum aeration and to disperse properly the colloidal structure of the dough so that a maximum amount of the gas will be retained.

The forces of gas production and of gas retention work simultaneously and integrate each other. Each strives for supremacy. If maximum gas production is reached before the colloidal elasticity of the dough is at an optimum, there is not enough gas left to aerate the mass when the elasticity peak is reached. Again if the dough reaches its maximum elasticity or gas retention peak before there is maximum gas production, much of the gas is lost. There is a peak of gas generation and a peak of gas retention. The ideal adjustment of all forces would cause the gas production peak to be reached in the same length of time from the start of fermentation as the gas retention peak. When both peaks are reached at the same time there frequently is combined in one loaf the largest volume together with the best grain, texture, crust color and other loaf characteristics which the flour in question will produce.

There is usually a range of fermentation time over which the forces of gas production are in balance with the forces of gas retention, during which period good bread or the desired product can be produced. It is reasonable to call this range in fermentation time the fermentation tolerance of the dough. As has been pointed out above, this range is dependent upon the balancing of all the forces of gas production against all the forces of gas retention. It is, therefore, apparent that fermentation tolerance is a resultant of many forces and of many tolerances and is frequently measured in units of time.

If a fermentation time is selected which is shorter than the fermentation tolerance, the bread is inferior. The same is true if a fermentation period is selected which is longer than the fermentation tolerance. Some flours and their doughs when handled in certain ways have very narrow ranges of fermentation tolerance. These same flours and doughs when handled in other ways or under formula adjustments may display a wide range of fermentation tolerance.

The baker strives so to regulate the factors of gas production and gas retention that they will produce their respective peaks simultaneously, thus combining into his bread the largest volume with the best grain, texture and other physical characteristics which the flour can produce. Fermentation is over when the dough enters the oven. Because of this fact the baker not only gains considerable information on the proper adjustment of the gas production and gas retention forces by the action of the dough during pan proof, but he also frequently uses different pan-proof times as a test of the correct fermentation.

Baking is the last step in the bread-making process. Many reactions occur in the oven. The test for the close of this period is a thoroughly baked loaf of bread. The ultimate goal of all three steps in bread production is good bread that measures up to the desired standard.

VARIATION IN CAROTINOID PIGMENT CONCENTRATION AMONG INBRED AND CROSSBRED STRAINS OF CORN¹

I. J. JOHNSON AND ELMER S. MILLER²

University of Minnesota, St. Paul, Minnesota

(Read at the Annual Meeting, May 1937)

The present-day method of corn improvement based upon the production of relatively homozygous inbred strains by controlled pollination followed by their recombination into hybrids offers exceptional opportunities for studies of variability in chemical composition. The inbred strains thus developed, as well as the hybrids between them, show wide differences in morphological characters. Many studies have been previously reported on the extent of variation in morphological characteristics among inbred strains and their hybrids but few studies have been made on the physiological differences between inbred or crossbred strains of corn.

Since inbred strains show striking differences in intensity of yellow endosperm color, the present study was made to determine the range in concentration of carotinoid pigments in the endosperm and to determine the extent to which variations in endosperm color were associated with variations in carotinoid pigment concentration. Analyses were also made to learn if differences in carotinoid pigment concentration in the endosperm might also be reflected in the leaf tissue of young plants grown under greenhouse conditions.

Experimental Material and Methods

The study on the variation in carotinoid pigments in corn was made on 19 inbred lines selected to represent a wide range in yellow endosperm color. The lines used had been inbred from 6 to 14 years and were homozygous for their respective color differences. Approximately 10 grams of shelled corn were ground in a Wiley mill, the pigments extracted with acetone and transferred to an ether solution in a separatory funnel.

In the comparison of carotinoid pigments in the endosperm and in young plants, four pairs of lines resulting from crosses of yellow and white endosperm parents were used. The lines had been maintained

¹ Contribution from the Division of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota. Paper No. 1532 of the Journal Series, Minnesota Agricultural Experiment Station. Also presented at the twenty-third annual meeting of the American Association of Cereal Chemists at Minneapolis, May 27-28, 1937.

² Assistant Professor of Agronomy and Plant Genetics, and Instructor, Department of Botany, respectively.

in a heterozygous condition for yellow endosperm during the inbreeding period, and subsequently purified for yellow and colorless endosperm. Each pair of lines was then relatively similar for all other genetic characters and differed only in endosperm color. Leaf pigment concentration was determined on the young plants grown in the greenhouse when they were 12 to 15 inches tall.

In another experiment, suitable crosses were made to provide endosperm genotypes of YYY , YYy , Yyy , and yyy , to study the relation between the number of dominant genes for yellow endosperm and the concentration of carotinoid pigments in the endosperm and in seedling plants.

The concentration of beta carotene,³ total carotinoids and chlorophyll was determined spectrophotometrically by the method described by Burr and Miller (1937).

Experimental Results

The data obtained in this study will be presented under three parts, variation in carotinoid pigments in the endosperm of inbred lines of corn, the relation between the number of dominant genes for yellow endosperm color to the concentration of carotinoid pigments, and the relation between carotinoid pigments in the endosperm to that in young corn plants.

Variation in Carotinoid Pigment Concentration Among Inbred Lines

Among the many yellow endosperm lines produced by inbreeding corn may be found a wide range in color from pale to very dark yellow. To determine the extent to which variations in degree of yellow endosperm color influenced the amount of carotinoid pigments, a group of 19 lines was selected which covered the maximum range available among inbred lines used in the corn improvement program. The seed was grown in 1935 and was fully matured and normal. Among the 19 lines, six were classified as dark yellow, seven medium yellow, and six as light yellow in color. The distribution of the lines in percentage of total carotinoid pigments is given in Table I.

The data given in Table I show that inbred lines may exhibit wide differences in percentage of carotinoid pigments, the 19 lines used in this study ranging from 0.0020 to .00158 in per cent of total carotinoid pigment. From the standpoint of differences in nutritional value, with particular reference to vitamin A, a knowledge of variability in carotene may be of great importance in breeding for improvement in feeding qualities. The wide differences obtained with the limited number of lines used suggest that a more extensive survey and study should be made among inbred strains of yellow corn to determine the possibilities of improvement in this respect.

³ Beta carotene refers to that fraction soluble in ligroin and insoluble in 92% methanol.

TABLE I

VARIATION IN CAROTINOID PIGMENTS IN THE GRAIN OF 19 INBRED STRAINS OF CORN

Range in total carotinoid %	Number of lines
.0001-.00030	1
.00031-.00050	4
.00051-.00070	3
.00071-.00090	1
.00091-.00110	3
.00111-.00130	5
.00131-.00150	1
.00151-.00170	1

The 19 lines were placed in three groups for intensity of endosperm color and the averages for total carotinoid pigments and range of variability determined (see Table II).

TABLE II

RELATION BETWEEN INTENSITY OF YELLOW ENDOSPERM COLOR IN CORN AND THE PERCENTAGE OF TOTAL CAROTINOID PIGMENTS

Endosperm color	Number of lines	Average percentage total carotinoids	Range
Dark yellow	6	.000869	.000443-.00121
Medium yellow	7	.000991	.000389-.00137
Light yellow	6	.000609	.000202-.00158

The lack of a strong relationship between the intensity of yellow endosperm color and the percentage of total carotinoids may be explained in part on the basis that many of the dark and medium yellow endosperm lines also carry genes for brown aleurone. The combined effect of the genes for yellow endosperm with those for brown aleurone usually results in a darker yellow color than those having the genes for yellow endosperm only. The light yellow lines averaged lowest in total carotinoids. These results would indicate some relation between total carotinoids in the endosperm and in endosperm color although dark yellow endosperm lines may not necessarily have a higher percentage of carotinoid pigments than light yellow endosperm lines.

Relation Between the Number of Dominant Genes for Yellow Endosperm and Carotinoid Pigments in the Endosperm

In feeding studies with homozygous yellow, heterozygous yellow, and homozygous white kernels on ears segregating for endosperm color, Hague and Trost (1928) have shown that vitamin A is transmitted exclusively with yellow endosperm color. No transfer of genes for vitamin A from the yellow parent to white kernels on segregating ears was found since white kernels on segregating ears were not more effec-

tive in promoting growth in rats than the white kernel parent used in the cross. Steinback and Boutwell (1920) had previously shown that white endosperm maize varieties were deficient in vitamin A while yellow endosperm varieties gave normal growth in rats. Yellow kernels selected from variegated maize gave better growth than white or pale yellow kernels.

From data published by Mangelsdorf and Fraps (1931) it was shown that the amount of vitamin A in corn was nearly directly proportional to the number of dominant genes for yellow endosperm color.

Suitable pollinations were available by which the four possible classes of endosperm genotypes *YYY*, *YYy*, *Yyy*, and *yyy* could be analyzed for beta carotene and total carotinoid pigments. The young plants from the four endosperm genotypes were grown in the greenhouse and analyzed for total carotinoids and for total chlorophyll. The data obtained in this study are given in Table III.

TABLE III

RELATION BETWEEN THE NUMBER OF DOMINANT GENES FOR YELLOW ENDOSPERM IN CORN TO THE CONCENTRATION OF CAROTINOID PIGMENTS IN THE ENDOSPERM AND IN THE LEAF TISSUE OF YOUNG PLANTS

Endosperm genotype	Carotinoid pigments in the endosperm				Total carotinoids in young plants	Total chlorophyll in young plants
	Total carotinoids		Beta carotene			
	%	Ratio	%	Ratio	%	%
YYY ¹	.000465	3.3	.000131	3.1	.01206	.0990
YYy	.000282	2.0	.000079	1.9	.01105	.0677
Yyy	.000139	1.0	.000042	1.0	.01305	.0713
yyy	.000042	0.3	.000011	0.3	.00600	.0487

¹ Average of two strains.

The data given in Table III show that the percentage of total carotinoids and beta carotene is approximately in direct proportion to the number of dominant *Y* genes. The ratios of 3.3, 2.0, and 1.0 for total carotinoid and 3.1, 1.9, and 1.0 for beta carotene correspond very closely with 3, 2, and 1 dominant *Y* genes. Colorless endosperm kernels contain only a very small amount of carotene. These results obtained by a physical method of analysis agree very closely with the results obtained from feeding experiments reported by Mangelsdorf and Fraps (1931).

Relation Between Carotinoid Pigment Concentration in the Endosperm to Carotinoid Pigment Concentration in Young Plants

The data in Table III suggested that seedlings grown from homozygous yellow endosperm kernels were not greatly different in con-

centration of carotinoid pigments than those grown from kernels heterozygous for yellow endosperm. The seedlings from the homozygous yellow kernels contained .01206% total carotinoids and the seedlings from heterozygous kernels .01205% total carotinoids (average of the two classes of heterozygous endosperms). The percentage of total carotinoids in the young plants from homozygous white kernels was found to be approximately one-half as great as from the yellow kernels. To study this relationship further, the seedlings from four pairs of yellow and white endosperm sister lines were analyzed for total carotinoids. These four pairs of inbred lines were maintained in a heterozygous condition for endosperm color during the period of inbreeding. Since the separation into homozygous yellow and white endosperm was not made until the lines were practically homozygous, these sister lines should be nearly identical for other genes. In this group of lines the percentage of carotinoid pigments in the grain was not determined, since previous analysis (see Table III) had shown that white endosperm lines were very low in carotinoid pigments. The average percentage of carotinoid pigments and total chlorophyll for the four white and four yellow sister lines are given in Table IV.

TABLE IV

RELATION BETWEEN ENDOSPERM COLOR IN CORN AND THE CONCENTRATION OF CAROTINOID PIGMENTS AND TOTAL CHLOROPHYLL IN YOUNG PLANTS

Endosperm	Pigment concentration in young corn plants		
	Total carotinoids	Beta carotene	Total chlorophyll
	%	%	%
Yellow	.0083	.00277	.0845
White	.0118	.00361	.1134

The results given in Table IV indicate clearly that there is no apparent relation between the formation of carotinoid pigments in the endosperm and the development of carotinoid pigments in young plant tissue. Although the amount of carotinoids in the plants grown from colorless endosperm kernels is somewhat higher than from yellow endosperm kernels, these differences may be due in part to the small numbers of comparisons used in this study.

These results also show that the genotype which does not permit formation of any appreciable quantities of carotinoids in the endosperm has no inhibiting effect in the formation of carotinoids in young plant tissue. From the standpoint of nutritional value of young corn plants, with respect to vitamin A, it is evident that yellow and white corn varieties are of equal value.

From the data given in Table IV, there appears to be some indica-

tion of a positive relationship between the amount of chlorophyll and the amount of carotinoid pigments in corn leaves. The data in Table III also indicate that the concentration of these two groups of pigments is closely associated.

Unpublished data obtained by Burr and Miller⁴ show that when such physiological factors as light intensity and quality of light are varied, the total carotinoid concentration fluctuates with the total chlorophyll concentration.

The extent to which these two pigment classes are associated in mature corn plants is being studied at the present time and will be reported at a later date.

Summary

The mature grain from 19 inbred lines of corn varying from light to dark yellow endosperm color showed wide differences in percentage of total carotinoid pigments. The variation in pigment concentration was not closely associated with the intensity of yellow endosperm color.

A very close relationship was obtained between the number of dominant Y genes for yellow endosperm color and concentration of total carotinoids and beta carotene. The percentage concentration obtained was in direct proportion to the number of dominant genes.

Young corn plants from white and yellow endosperm sister lines were analyzed for chlorophyll and carotinoid pigments. The percentage of carotinoid pigments in leaf tissue from white endosperm lines was found to be slightly higher than from their yellow endosperm sister lines. The differences may be due to the small number of samples used. These results suggest that the formation of carotinoid pigments in the leaf tissue and that deposited in the endosperm are independent of each other.

A positive association was obtained between the percentage of chlorophyll and carotinoid pigments in corn leaf tissue.

Literature Cited

- Burr, George O., and Miller, Elmer S.
1937 Chlorophyll ratios and photosynthesis. (In press.)
Hague, Sigfred M., and Trost, John F.
1928 An inheritance study of the distribution of vitamin A in maize. *J. Biol. Chem.* 80: 107-114.
Mangelsdorf, P. C., and Fraps, G. S.
1931 A direct quantitative relationship between vitamin A in corn and the number of genes for yellow pigmentation. *Science* 73: 241-242.
Steinback, H., and Boutwell, P. W.
1920 Fat soluble vitamins. III. The comparative nutritive value of white and yellow maizes. *J. Biol. Chem.* 41: 81-96.

⁴University of Minnesota, Department of Botany.

THE AMYLASES OF DIFFERENT BARLEY VARIETIES¹

CLAUDE H. HILLS and C. H. BAILEY

Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

(Read at the Annual Meeting, May 1937)

Barley has maintained its position as an important cereal crop in no small measure because of its malting properties. It is well suited to the production of malt because the endosperm is covered with a husk which protects the growing acrospire and which also serves as a filtering medium during the brewing process. Furthermore, malted barley is high in diastatic activity and contains less undesirable protein fractions than other malted cereals.

Notwithstanding the interest in the problem of the enzymatic changes involved in malting, there have not been many studies reported concerning the diastatic activity of different American barley varieties. Dickson and coworkers (1935) made a comparative study of malting barleys grown in Wisconsin. They found that Manchuria, Velvet, and Oderbrucker yielded better quality malts than any of the other varieties studied. These varieties were also highest in diastatic activity.

Shellenberger and Bailey (1936a) made an extensive study of the more common barley varieties grown in Minnesota. They observed that Oderbrucker and Velvet were high and Peatland was low in diastatic activity. They also measured the dextrinizing (α -amylase) activity of the ungerminated barley, the green malt, and the dry malt of each variety. Their data indicated that ungerminated barley may contain a small but measurable amount of α -amylase activity.

Various other studies have presented conflicting conclusions as to the actual existence or absence of α -amylase in dormant mature cereals. Nordh and Ohlsson (1932) stated that α -amylase was entirely absent in dormant barley and that the diastase of the ungerminated kernels was pure β -amylase. The findings of Lüers and Rümmler (1933) and Chrzaszcz and Janicki (1933, 1936) supported Ohlsson's conclusion.

The work of Jozsa and Gore (1932) and of Gore and Jozsa (1932) indicated that α -amylase, as well as β -amylase, is present in normal wheat flour. Sandstedt, Blish, Mechem, and Bode (1937) state that

¹ Paper No. 1552, Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Claude H. Hills to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1937.

"the widespread belief that ungerminated grain contains only beta amylase, in contrast to germinated or malted grain, which contains both the alpha and beta forms, may be an erroneous one."

Materials and Methods

The barley used in this study was furnished by the Agronomy Division of the University of Minnesota from the increase plots at University Farm, St. Paul, Minnesota. The following eight varieties were obtained from the 1935 crop: Oderbrucker, Velvet, Trebi, Peatland, Glabron, Wis. No. 38, Spartan, and Minn. No. 462 (an unnamed variety). These last two varieties were not grown in the 1936 crop, so they were replaced by Odessa and Manchuria.

The samples from these two crop years afforded excellent material for making seasonal comparisons. The growing season of 1936 was extremely hot and dry and produced barley having small, shriveled kernels, high in protein content and diastatic activity. The season of 1935 was quite the reverse. Nearly optimum conditions prevailed during the growing season, resulting in barley having large, plump kernels that were high in starch and low in protein content.

Malting procedure: The barley samples were malted in the laboratory malting equipment designed and described by Shellenberger and Bailey (1936). Eight 300-gram samples of barley were placed in cheese-cloth bags and steeped in a large jar of water at 12.5° C. to 13.0° C. for 52 hours. Air was bubbled through the steep continuously and the temperature of the steep was controlled by a continuous stream of cold water entering the jar.

At the end of the steeping period the samples of barley were transferred to wire germinating cages and placed in the previously cooled and humidified malting cabinet. The barley was allowed to germinate at $16^{\circ} \pm .5^{\circ}$ C. until the acrospires of the grain attained the length of the kernel. This required approximately six days. A constant stream of humidified air was drawn through the cabinet and the cages were revolved one-quarter turn every four hours to insure uniform conditions of germination. On completion of germination the malt was dried inside the malting cabinet according to the following schedule: 12 hours at 30° C., 12 hours at 35° C., 12 hours at 40° C., 12 hours at 50° C., and 6 hours at 70° C.

Extraction of amylases: The fresh green malt was taken from the malting cabinet at the desired stage of germination and immediately stored in paper bags in a room maintained at -20° C. The frozen samples were ground through a small hand-driven food chopper operated in the refrigerated room. The dry malt samples, after having

the sprouts removed, were ground through two series of small rolls having 28 and 40 corrugations per inch respectively. Ungerminated barley was ground on the same set of rolls and to the same degree of fineness.

Water extracts of dry malt and ungerminated barley for the determination of diastatic activity were prepared as follows: 2.5 g. (dry basis) of ground barley or malt were extracted with 50 c.c. of water for 3 hours at 21° C., shaking the flask every 30 minutes. Then the extraction mixture was poured into a centrifuge tube and centrifuged at half speed for exactly 5 minutes.

Green malt extracts were more difficult to prepare. In order to insure adequate extraction of enzymes, it was necessary to grind the chopped green malt to the same degree of fineness as the ground barley and dry malt samples. This was accomplished by triturating 2.5 g. (dry basis) of chopped frozen green malt with 5 g. of clean crushed quartz and a little water in a mortar. The finely ground sample was washed into a flask with additional water to make a total of 50 c.c. of water added. The conditions of extraction were the same as for the barley and dry malt.

Extraction of "total" amylase: Water extracts of barley and malt were used to determine the "free" or water-soluble amylases. The extraction of "total" amylases was effected by partially digesting the sample with papain. To 50 c.c. of the water extraction mixture were added 250 mg. (.5%) of papain and the sample digested 24 hours at 21° C. The mixture was centrifuged and the supernatant liquid used for the determination of "total" α - and β -amylases. Hills and Bailey (1938) have shown that the "total" amylase activity of barley determined in this manner is equal to the sum of the water-soluble amylase and the amylase activity of the water-insoluble residue.

β -Amylase: All β -amylase determinations were made in the following manner: To 35 c.c. of 2% soluble starch solution containing 0.1 N acetate buffer (pH=4.65) was added a sufficient volume of 0.1 N acetate buffer (pH=4.65) to make the final volume (substrate + enzyme addition) equal 46 c.c. Then 2 c.c. of a 1:10 dilution of the original barley or malt extract were added and the mixture digested 1 hour at $30^{\circ} \pm .1^{\circ}$ C. At the end of 1 hour enzyme action was arrested by the addition of 2 c.c. of 10% H_2SO_4 and 2 c.c. of 12% Na_2WO_4 . The quantity of maltose produced was determined on a 5 c.c. aliquot by Blish and Sandstedt's (1933) modification of the Hagedorn and Jensen (1923) micro method for determining blood sugar.

α -Amylase: Alpha amylase activity was determined by a modification of the Wohlgemuth (1908) method. Progressively increasing

amounts of the barley or malt extract, suitably diluted, were added to a series of test tubes in an ice-water bath. Each tube contained 1 c.c. of 2% soluble starch buffered at pH = 4.65 with 0.1 N acetate buffer. Water was added to make the total volume equal 6 c.c., and the mixture digested 1 hour at $40^{\circ}\text{C} \pm .1^{\circ}\text{C}$. The tubes were removed, placed in an ice-water bath and 10 c.c. of ice-cold water and 1 drop of 0.1 N iodine solution were added to each tube. The end-point was the hue possessed by the tube, observed by artificial light, which most nearly matched the RV Normal tone of Chart D in Mulliken's book (1911). The number of cubic centimeters of 2% starch solution used in this instance (1 c.c.) divided by the fraction of a cubic centimeter of the original enzyme solution required, gives the Wohlgemuth value for the change of starch iodine color from blue to violet. This value accordingly expresses the equivalent number of cubic centimeters of 2% soluble starch solution dextrinized by 1 c.c. of the original enzyme extract in 1 hour at 40°C .

TABLE I

FREE AND TOTAL AMYLASE ACTIVITY OF UNGERMINATED BARLEY. BARLEY GROWN AT UNIVERSITY FARM, ST. PAUL

Laboratory number	Variety	Dry weight basis		Amylase activity		Ratio A ¹
		1000- kernel weight G.	Protein N $\times$ 6.25 %	Free	Total	
				Mg. maltose		
1935 Crop						
209	Oderbrucker	26.36	15.94	130	261	2.01
210	Velvet	26.17	16.44	132	257	1.95
211	Trebi	33.55	16.13	141	287	2.04
212	Peatland	23.17	17.69	83	285	3.43
213	Glabron	27.40	16.94	115	239	2.08
214	Wis. No. 38	27.73	16.19	112	210	1.88
215	Spartan	38.36	16.31	90	175	1.94
216	Minn. No. 462	24.73	15.81	96	185	1.93
	Average	28.43	16.43	112	237	2.16
1936 Crop						
201	Oderbrucker	14.55	20.00	168	359	2.14
202	Velvet	15.17	20.25	150	342	2.28
203	Trebi	18.80	22.81	225	485	2.16
204	Peatland	12.40	21.63	112	406	3.63
205	Glabron	18.30	19.56	152	303	1.99
206	Wis. No. 38	15.58	19.81	115	253	2.20
207	Odessa	14.63	21.13	157	373	2.38
208	Manchuria	14.01	20.88	162	430	2.65
	Average	15.43	20.76	155	369	2.43

¹ Ratio A = $\frac{\text{Total amylase activity of barley}}{\text{Free amylase activity of barley}}$

Free and Total Amylase Activity of Barley and Malt

It was desirable to measure not only the water-soluble amylases, but also the total amylase activity of each of the barley samples. Table I gives the free and total amylase activity of the 16 barley samples studied and the ratio between these two values. The protein content and 1,000-kernel weight are also given in order to compare these data with the amylase activity of each variety.

The diastatic activity of the 1936 samples is much greater than for the previous year's crop. There is also a large variation in protein content and 1,000-kernel weight for the same varieties from different crop years. The barley samples grown in 1936 are higher in protein content and lower in 1,000-kernel weight than the same varieties from the 1935 crop.

The varieties Peatland and Trebi were consistently high in total amylase for both years. Manchuria, Velvet, and Oderbrucker were also high in diastatic activity. Wis. No. 38, Spartan, and Minn. No. 462 are relatively low in diastatic activity. There are no consistent varietal differences in protein content for both years.

The increase in amylase activity by papain extraction is indicated in column 7 as the ratio between total and free amylase activity. In general, this increase ranges from 100% to 130%. Peatland shows an abnormally high proportion of bound amylase. There is very little correlation between protein content or 1,000-kernel weight and the ratio of total to free amylase activity.

The amylase activity of green malt and dry malt is recorded in Table II.

The varietal and seasonal differences observed in amylase activity of ungerminated barley appear also in the green malt and dry malt. There is excellent agreement between the total amylase activity of barley before and after malting as evidenced by the constancy of Ratio B. The correlation between total amylase activity of ungerminated barley and of green malt is highly significant ($r = +0.991 \pm 0.003$). This would indicate that the total β -amylase activity of ungerminated barley is an accurate criterion for predicting the total β -amylase activity of malted barley. The increase in total β -amylase activity on malting was 25% to 29%. Myrbäck and Myrbäck (1936) observed a 30% increase in total amylase on germination of barley.

Ratio C indicates that the per cent of total malt amylase of green malt that is water-soluble is 66% and 75% respectively for the two crop years. The increase in the proportion of water-soluble amylase during germination is undoubtedly due to the action of proteolytic enzymes. There is a slight decrease of approximately 16% to 19% in total β -amy-

TABLE II
 AMYLASE ACTIVITY OF GREEN MALT AND DRY MALT. BARLEY GROWN AT
 UNIVERSITY FARM, ST. PAUL

Laboratory number	Variety	Amylase activity of green malt			Ratio C ²	Amylase activity of dry malt		
		Free	Total	Ratio B ¹		Free	Total	Ratio D ³
		Mg. maltose				Mg. maltose		
1935 Crop								
209	Oderbrucker	263	358	1.37	.73	248	302	1.19
210	Velvet	218	320	1.25	.68	220	285	1.12
211	Trebi	238	368	1.28	.65	240	320	1.15
212	Peatland	220	355	1.25	.62	230	327	1.09
213	Glabron	187	276	1.15	.68	185	225	1.23
214	Wis. No. 38	163	265	1.26	.62	182	228	1.16
215	Spartan	125	210	1.20	.60	140	178	1.18
216	Minn. No. 462	155	233	1.26	.67	187	203	1.15
	Average	196	296	1.253	.656	204	259	1.16
1936 Crop								
201	Oderbrucker	381	457	1.27	.83	328	388	1.18
202	Velvet	363	437	1.25	.85	318	363	1.18
203	Trebi	416	631	1.30	.66	394	493	1.28
204	Peatland	353	497	1.22	.71	375	418	1.19
205	Glabron	292	382	1.26	.76	263	325	1.18
206	Wis. No. 38	228	315	1.25	.72	228	283	1.11
207	Odessa	370	515	1.38	.72	335	431	1.19
208	Manchuria	410	573	1.33	.72	363	474	1.21
	Average	352	475	1.283	.746	326	397	1.19

$$^1 \text{ Ratio B} = \frac{\text{Total amylase of green malt}}{\text{Total amylase of barley}}$$

$$^2 \text{ Ratio C} = \frac{\text{Free amylase of green malt}}{\text{Total amylase of green malt}}$$

$$^3 \text{ Ratio D} = \frac{\text{Total amylase of green malt}}{\text{Total amylase of dry malt}}$$

lase activity of malt on drying, presumably due to heat inactivation. The difference between free and total amylase activity decreased during germination and continued to decrease during drying of the malt.

The varieties that are high in diastatic activity for the season of 1935 are likewise high for the season of 1936. Trebi, Peatland, and Manchuria are consistently high in β -amylase activity and Wis. No. 38 is consistently low for both years.

α -Amylase of Barley and Malt

Jozsa and Gore (1932), and Gore and Jozsa (1932) have demonstrated that normal wheat flour may contain a measurable quantity of α -amylase. Shellenberger and Bailey (1936) found variations in the

starch dextrinizing activity of 26 barley samples, although in most instances the activity was very low. These observations do not agree with Nordh and Ohlsson's (1932) conclusion that ungerminated barley contains no α -amylase activity.

An attempt was made to vary the activity of the two amylase functions of an extract of ungerminated barley using Ohlsson's (1926) technique for separating α - and β -amylases.

Solution A: 2.5 g. (dry basis) of Velvet barley were extracted with 50 c.c. of water and the extract diluted 1:10 with water.

Solution B: 25 c.c. of the original barley extract were acidified with 1.25 c.c. of 0.1 N HCl and kept at 0–5° C. for 15 minutes (pH 3.35). Then .75 c.c. of 0.2 M Na_2HPO_4 was added and 2.7 c.c. of this solution (pH 6.67) diluted to 25 c.c. with water.

Solution C: 20 c.c. of the original barley extract were heated to 60° C. for 15 minutes, cooled, and diluted 1:10 with water.

Acidification to pH 3.3 destroys most of the α -amylase activity with very little reduction in β -amylase, whereas heating to 60–70° C. impairs β -amylase more than α -amylase.

TABLE III
DETERMINATION OF α -AMYLASE IN UNGERMINATED BARLEY

Enzyme solution	Mg. maltose per 50 c.c.	Wohlgemuth values
Sample No. 202 Velvet 1936 Crop		
2 c.c. barley extract (Solution A)	150	.33
2 c.c. solution B	122	.33
Sample No. 210 Velvet 1935 Crop		
2 c.c. barley extract (Solution A)	105	1.25
2 c.c. solution B	95	.13
2 c.c. solution C	34	1.00

The sample of Velvet from the 1935 crop showed a separation of its α - and β -amylase functions by either acid (Solution B) or heat treatment (Solution C). The fact that it was possible to vary the two amylase functions independently of each other constitutes evidence that both α - and β -amylases are present in ungerminated barley.

The sample of ungerminated barley from the 1936 crop appears to contain no α -amylase activity. The acid treatment, which destroys α -amylase chiefly, caused no appreciable reduction in the Wohlgemuth value. The low starch-dextrinizing activity (0.33) of this sample was apparently due to the hydrolytic action of β -amylase. For this sample the starch-iodine color changed from blue to light blue to faint blue, or

colorless, whereas, for the sample No. 210, which contained a small amount of α -amylase, there was a distinct transition from blue to violet to red.

The α -amylase activity of ungerminated barley, green malt, and dry malt is given in Table IV.

TABLE IV
 α -AMYLASE ACTIVITY OF BARLEY AND MALT. BARLEY GROWN AT UNIVERSITY FARM, ST. PAUL

		C.c. of 2% soluble starch solution dextrinized per c.c. of barley extract on 1 hr. digestion, 40° C.				
Number	Variety	Barley meal	Green malt		Dry malt	
		Free	Free	Total	Free	Total
1935 Crop						
209	Oderbrucker	1.50	60	125	100	130
210	Velvet	1.25	85	125	86	107
211	Trebi	.71	44	103	86	94
212	Peatland	3.08	65	120	111	115
213	Glabron	1.00	41	107	79	103
214	Wis. No. 38	.50	33	86	65	100
215	Spartan	.50	28	63	54	65
216	Minn. No. 462	1.20	36	73	70	88
Average		1.22	49	100	81	100
1936 Crop						
201	Oderbrucker	.42	104	143	125	125
202	Velvet	.33	86	139	119	125
203	Trebi	.41	81	154	114	119
204	Peatland	.48	93	161	147	128
205	Glabron	.44	81	104	100	139
206	Wis. No. 38	.38	61	106	91	122
207	Odessa	.38	114	200	135	200
208	Manchuria	.40	109	161	156	169
Average		.41	81	146	123	141

The barley samples from the 1935 crop contain a significant quantity of α -amylase, with the possible exception of the varieties Wis. No. 38 and Spartan. The green malt and dry malt from the 1935 crop are much lower in α -amylase activity than those from the following year. There was a slight increase in free α -amylase on drying of the malt but the total α -amylase activity changed very little, if any.

The barley samples grown in 1936 were very low in dextrinizing activity, the Wohlgemuth values ranging from .33 to .48. For each sample the starch-iodine color change was from blue to faint blue or colorless. Thus it is concluded that the samples of barley from the 1936 crop contain no α -amylase and that the loss of blue starch-iodine color was almost entirely due to the action of β -amylase.

The varieties, Odessa, Manchuria, Oderbrucker, and Peatland are high in α -amylase activity and Wis. No. 38 is low. There is a significant positive correlation between the total α - and total β -amylase activity of green malt ($r = 0.869 \pm 0.041$).

Effect of α -Amylase Upon Saccharifying Activity of Green Malt

As was previously pointed out in this discussion, the increase in total β -amylase activity on germination of barley, as measured by maltose production, is approximately 25% to 29%. Klinkenberg (1932) observed that α -amylase acting on soluble starch hydrolyzed 36% of the starch to maltose. Therefore, it is conceivable that part of the increase in saccharifying activity on germination of barley may be due to the action of α -amylase appearing upon germination.

Freeman and Hopkins (1936) studied the rate of hydrolysis of soluble starch by α - and β -amylases acting separately and the combined effect of the two acting together. They found that in the earlier stages of hydrolysis, α -amylase produced almost as much maltose as did an equivalent amount of β -amylase. A mixture of α - and β -amylase in low concentration functioned additively in hydrolyzing starch but when the ratio of α - to β -amylase was low the combined rate was greater than the sum of the two component rates.

These results would suggest that the increase in total β -amylase activity (maltose production) on germination is partly due to the effect of α -amylase and that the β -amylase concentration may increase very little, if any. In order to test this explanation, a solution of purified α -amylase was added to a water extract of Velvet (1936) barley, which was observed to be free of β -amylase, and the increase in maltose production noted.

The purified α -amylase preparation used was prepared by Olof Stamberg, of the Division of Agricultural Biochemistry, University of Minnesota. A solution was prepared by triturating 50 mg. of dry preparation with a few cubic centimeters of water in a mortar and diluting to 50 c.c. volume. This solution was further diluted 1:5 with water so that the final concentration was 0.20 mg. per cubic centimeter. The ratios of β - to α -amylase employed are in the range of the observed ratio of the two enzymes in the green malt samples.

The ratios of β - to α -amylase of the three solutions examined are given in the last column of Table V. The solutions of purified α -amylase produced an appreciable quantity of maltose. The amount of maltose produced by α - and β -amylases acting together was approximately the sum of the two amounts produced by their acting separately.

By using the data in Table V together with the values for the average

TABLE V
EFFECT OF α -AMYLASE UPON MALTOSE PRODUCTION BY β -AMYLASE

Enzyme addition	C.c. 2% starch dextrinized by α -amylase	Mg. maltose	Increase due to α -amylase		Ratio β/α
			Mg. maltose	%	
2.1 c.c. α -amylase solution	6.0	17			
2.8 c.c. α -amylase solution	8.0	21			
4.2 c.c. α -amylase solution	12.0	29			
1.6 c.c. of β -amylase solution		124			
1.6 c.c. β + 2.1 c.c. α	6.0	142	18	14.52	20.67 : 1
1.6 c.c. β + 2.8 c.c. α	8.0	151	27	21.77	15.50 : 1
1.6 c.c. β + 4.2 c.c. α	12.0	162	38	30.64	10.33 : 1

α - and β -amylase activities of the samples from the two crops, it was possible to calculate the increase in maltose production due to the increase in α -amylase activity on germination. These calculations are shown in Table VI. This calculated increase was compared with the observed increase in saccharifying activity.

TABLE VI
CALCULATED INCREASE IN SACCHARIFYING ACTIVITY DUE TO α -AMYLASE APPEARING ON GERMINATION. BARLEY GROWN AT UNIVERSITY FARM, ST. PAUL

Number		Average 1935 crop	Average 1936 crop
1	Total β -amylase activity of barley (per .2 c.c.)	237	369
2	Total β -amylase activity of green malt (per .2 c.c.)	296	475
3	Apparent increase on germination. No. 2/No. 1	24.89%	28.73%
4	Total α -amylase activity of green malt (per 1.0 c.c.)	100	145
5	Total α -amylase activity per .2 c.c.	20	29
6	Ratio $\frac{\text{Total } \beta \text{ of barley}}{\text{Total } \alpha \text{ of malt}}$	11.85 : 1	12.72 : 1
7	Calculated increase in maltose production by addition of α -amylase to β -amylase in the ratio found in germinated barley (No. 6) By interpolating from values in Table V.	27.53%	26.50%

The calculated increase in maltose production shown in Table VI was obtained by interpolating between the values given in Table V for the relationship between the ratio of β - and α -amylases and the per cent increase in maltose production. This calculated increase in β -amylase activity (maltose production) agrees closely with the actual observed increase. These results lead to the conclusion that the concentration of total β -amylase of barley does not change on germination and that the

apparent increase in β -amylase activity, as measured by the rate of maltose production, is due, in reality, to the increase in α -amylase activity.

Summary

The barley samples grown in 1936, an abnormally dry year, were much higher in protein content and lower in 1,000-kernel weight than the same varieties grown in 1935, a season having better growing conditions. The diastatic activity of the barley and malt from the 1936 crop was approximately 50% higher than that from the previous year's crop.

Contrary to the opinion of various investigators, sound ungerminated barley may contain a measurable amount of α -amylase. The barley samples from the 1936 crop were lacking in α -amylase but most of the samples grown in 1935 contained appreciable quantities of α -amylase.

A high positive correlation was observed between the total β -amylase activity of barley and of green malt, which suggests the possibility of using the β -amylase activity of a papain digest of barley to predict the β -amylase activity of the malted grain. There was also a significant positive correlation between total α -amylase and total β -amylase of green malt.

A comparison of the barley varieties in regard to both diastatic functions showed that Odessa, Manchuria, and Oderbrucker were high, and Wis. No. 38, Spartan, Minn. No. 462, and Glabron were low. Trebi was highest in β -amylase but was deficient in α -amylase activity.

Germination of barley resulted in a relatively large increase in free (water-soluble) β -amylase activity but the increase in total β -amylase activity was only 25% to 29%. However, it was found that the concentration of total β -amylase did not change on germination of barley and that the observed increase in total β -amylase activity, as measured by the rate of maltose production, was due to the increase in α -amylase activity.

Literature Cited

- Blish, M. J., and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* 10: 189-202.
- Chrzaszcz, T., and Janicki, J.
1933 Die wirkliche und scheinbare Amylasemenge in verschiedenen Getreidearten vor und während der Keimung. *Biochem. Z.* 265: 260-281.
- 1936 Amylase during the growth and ripening of grains. *Biochem. J.* 30: 1298-1302.
- Dickson, J. G., Shands, H. L., Dickson, A. D., and Burkhart, B. A.
1935 Barley and malt studies I. Developing new varieties of barley for malting and their properties. *Cereal Chem.* 12: 596-609.
- Freeman, G. C., and Hopkins, R. H.
1936 The mechanism of degradation of starch by amylases. *Biochem. J.* 30: 446-450.

- Gore, H. C., and Jozsa, S.
1932 Sugar formation by diastatic enzymes of flour. *Ind. Eng. Chem.* **24**: 99-102.
- Hagedorn, H. C., and Jensen, B. N.
1923 Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. *Biochem. Z.* **135**: 46-58.
- Hills, Claude H., and Bailey, C. H.
1938 The nature of the increase in amylase activity of germinating barley. *Cereal Chem.* **15**: 273-281.
- Jozsa, S., and Gore, H. C.
1932 Development of diastatic enzymes of malt and flour. *Ind. Eng. Chem.* **24**: 95-98.
- van Klinkenberg, G. A.
1932 Über die Spezifität der Amylasen. *Z. Physiol. Chem.* **212**: 173-195.
- Lüters, H., and Rümmler, W.
1933 Development of amylases during the germination of barley. *Wochschr. Brau.* **50**: 297-301. *Abstr. in Chem. Abstr.* **28**: 3171 (1934).
- Mulliken, S. P.
1911 Identification of pure organic compounds. Vol. III. John Wiley and Sons, New York.
- Myrbäck, K., and Myrbäck, S.
1936 Über freie und gebundene Amylase in der Gerste. *Biochem. Z.* **285**: 282-289.
- Nordh, G., and Ohlsson, E.
1932 Amylasen in ruhenden und keimenden Samen. I. Gerste. *Z. Physiol. Chem.* **204**: 89-100.
- Ohlsson, E.
1926 On the two components of malt diastase. *Compt. rend. trav. lab. Carlsberg.* **16**: No. 7, 1-68.
- Sandstedt, R. M., Blish, M. J., Mecham, D. K., and Bode, C. E.
1937 Identification and measurement of factors governing diastasis in wheat flour. *Cereal Chem.* **14**: 17-34.
- Shellenberger, J. A., and Bailey, C. H.
1936 Design and operation of laboratory malting equipment. *Brewers Tech. Rev.* **11**: 3-5.
1936a Biochemical distinctions between barley varieties. *Cereal Chem.* **13**: 631-655.
- Wohlgemuth, J.
1908 Über eine neue Methode zur quantitativen Bestimmung des diastatischen Ferments. *Biochem. Z.* **9**: 1-9.

SOME OBSERVATIONS ON MALTOSE PRODUCTION IN FLOUR AND DOUGH¹

E. A. FISHER, P. HALTON, and S. F. HINES

The Research Association of British Flour-Millers, St. Albans, England

(Received for publication October 27, 1937)

It is well known that more than one kind of sugar is normally present in flour and that sugar production in dough continues during fermentation.

Cane sugar is present in all wheat flours sometimes to the extent of 2 to 3%. Reducing sugars are present in very small quantities only. During panary fermentation sugars of the maltose type must be produced because such sugars only are directly acted upon by yeast with the production of carbon dioxide. Without such sugars, or with an inadequate supply, insufficient gas would be produced by the yeast, especially in the critical or proving period of fermentation, and only small, heavy, stodgy loaves would result.

Production of sugars during fermentation takes place in two ways: (1) from the starch by the action of *diastase* present in the *flour*, and (2) from the cane sugar present through the action of *invertase* present in the *yeast*. Thus, flour diastase converts some starch into maltose which is then converted into glucose by the maltase present in yeast. On the other hand, invertase present in yeast will convert cane sugar into glucose and fructose which may then be converted into alcohol and carbon dioxide by the zymase of the yeast.

As is well known, flours vary widely in gas-producing capacity. Some produce a steady and large supply of gas throughout the whole fermentation period. Others will produce a steady but smaller supply. Others again may gas freely in the earlier stages of fermentation but gas production may slow up later, so that in the critical or proving period of fermentation insufficient gas may be produced to properly inflate the dough, small, stodgy loaves resulting.

These differences in rate of gas production are due to several factors, one of which is the variation that exists in the *diastatic capacity* of different flours. The greater the diastatic capacity the greater will be the rate of production of maltose from starch, and the greater, therefore, will be the production of carbon dioxide from maltose by

¹ First issued for private circulation as Confidential Report No. 13 of the Research Association of British Flour-Millers, December, 1929. Released for publication by the Council of the Research Association, November, 1935.

the yeast. If the diastatic capacity of a flour is too low, gas production may still be rapid in the earlier stages of fermentation because this early gas is produced from the sugars already existing in the flour. When this pre-formed sugar in such low diastatic flours has been used up, the rate of gas production will fall rapidly and may become entirely inadequate in final proof. In such cases gas production during final proof may be maintained at a high and satisfactory rate by increasing the diastatic power of the flour by the addition to the flour of small quantities (*e.g.*, 0.1% = $4\frac{1}{2}$ ounces per sack of 280 pounds) of highly diastatic malt extract. Gas production can be increased still further in some cases by adding with the malt extract a further 0.05% (or $2\frac{1}{4}$ ounces per sack) of ammonium phosphate. This is illustrated in

TABLE I

EFFECT OF MALT EXTRACT AND AMMONIUM PHOSPHATE ON RATE OF GAS PRODUCTION OF VARIOUS FLOURS

50 g. flour, 1 g. (= 2%) yeast, 0.625 g. (= $1\frac{1}{4}$ %) salt, 30 c.c. water, and temperature of fermentation of 80° F.

	No. 2 N. Manitoba		Garnet		No. 1 Hard Winter	
		M.E. ¹ +A.P.		M.E. ¹ +A.P.		M.E. ¹ +A.P.
	C.c.	C.c.	C.c.	C.c.	C.c.	C.c.
1st hour.....	31	41	30	47	43	30
2nd ".....	78	84	72	79	74	67
3rd ".....	89	102	83	102	83	83
4th ".....	106	121	106	122	97	101
5th ".....	108	127	71	137	113	116
6th ".....	82	117	34	93	106	120
7th ".....	51	90	24	50	55	103
8th ".....	36	53	16	37	35	56
Total gas in 24 hours..	840	1099	516	983	870	1000

¹ 0.1% malt extract and 0.05% ammonium phosphate.

Table I and Plate I. It will be noticed from Table I that, with 2% yeast and at 80° F., the No. 1 Hard Winter flour gassed freely and at an increasing rate for 6 hours, the No. 2 N. Manitoba for 5 hours, and the Garnet flour for only 4 hours. With malt extract and ammonium phosphate present rate of gas production was not materially increased in the first two hours, but was increased in the third and subsequent hours, and the high rate was maintained for 6 or 7 hours. This effect was most marked with Garnet flour, the poorest "gasser" of the three, and least with Hard Winter, the best gasser.

The Manitoba and Garnet flours were baked using the same formula of 2% yeast, $1\frac{1}{4}$ % salt, fermentation temperature 80° F., and

A1.	Manitoba, without malt extract, 4	hours to oven.							
A2.	"	"	"	"	"	"	"	"	"
A3.	"	"	"	"	"	"	"	"	"
B1.	"	with malt extract and ammonium phosphate, 4	hours to oven.						
B2.	"	"	"	"	"	"	"	"	"
B3.	"	"	"	"	"	"	"	"	"
C1.	Garnet, without malt extract and ammonium phosphate, 4	"	"	"	"				
C2.	"	"	"	"	"	"	"	"	"
C3.	"	"	"	"	"	"	"	"	"
D1.	"	with malt extract and ammonium phosphate, 4	hours to oven.						
D2.	"	"	"	"	"	"	"	"	"
D3.	"	"	"	"	"	"	"	"	"

It will be seen from Plate 1 how closely similar in volume are A1 and B1, and C1 and D1; that is, where gas production is already adequate malt extract may have little effect on loaf volume. When gas production is deficient malt extract, especially with the addition of ammonium phosphate, may produce a large effect on loaf volume, the effect being the greater the greater the gas deficiency. The loaves of the C series decreased greatly in volume from C1 to C3 whereas the D series actually increased in volume from D1 to D3. The Garnet flour benefited more than the Manitoba through the addition of malt extract.

It is obvious that no conclusions whatever concerning the relative baking qualities of Garnet and Manitoba, or for that matter of any series of flours, can be drawn unless and until adequate gas production is assured in each case. In all experimental work a naturally poor or unsatisfactory "gasser" must be corrected, as far as possible, by treatment with malt extract and ammonium phosphate.² In commercial practice inadequate gas production in some flours (such as certain Plate, English and some American wheats) is corrected by blending with exceptionally good gassers, such as durum or low grade Manitobas.

The importance of gas production as a separate factor in the quality problem arose out of the work of Wood (1907). Methods of controlling it by the addition of malt extract and ammonium phosphate and in other ways were subsequently worked out by Humphries who has emphasized the supreme practical importance of the point in the numerous Reports of the Home Grown Wheat Committee of the National Association of British and Irish Millers from 1907 onwards.

Two very extensive investigations on the diastatic capacity of flours and its relation to the "strength" problem were carried out by Rumsey (1922) and by Collatz (1922). These workers added considerably to our knowledge of the problem and Rumsey developed a method of estimating the rate of formation of maltose in a flour. For further work on this problem see also Jørgensen (1931), Blish, Sand-

² Two per cent. of cane sugar will have a similar effect.

stedt, and Astleford (1932), Steller, Markley, and Bailey (1935) and Landis and Frey (1936). A modification of this method introduced by Kent-Jones (1924), although less exact than the original method, is simple in technique, can be carried out by a comparatively inexperienced worker, and has proved itself to be of considerable value in the routine examination of flour.

The method consists of mixing 30 g. of flour with 160 c.c. of water, keeping the mixture at 27° C. for one hour, and then determining the amount of reducing sugar present in the mixture. The figure obtained is called the *maltose figure* and is a measure of the amount of maltose produced, in the particular conditions of the experiment, in one hour together with the small but variable amount of maltose initially present in the flour. The latter is usually so small that it can be ignored without impairing the practical value of the determination.

From what has been written above concerning the importance of maltose production as a factor in gas production in doughs, it might at first appear that the maltose figure should afford a measure of gas production also, in which case the long, tedious and cumbersome gassing test could be replaced by the short and simple maltose test. Kent-Jones (1924) indeed is of this opinion for he states:

"There is a definite connection between the maltose figure and gas production. Flours which give high maltose figures give great gas production, and moreover, still continue to give large quantities of gas after some hours. Those flours which have . . . low maltose figures may give in the initial stages a sufficiency of gas, but fail to do so later on at the critical period."

Kent-Jones recommends that the maltose test should be used as a substitute for the gas test, but the very few data available in the literature are altogether insufficient to establish the relationship claimed. There are other factors, besides maltose production, that contribute to adequate gas production in doughs, and in point of fact it has been found in the writers' laboratories that many flours of relatively low maltose figure behave satisfactorily in the bakehouse as regards gas production.

In an attempt to ascertain the precise relation between maltose figure and gas production, a large number of determinations of both have been made on a variety of flours and the results examined statistically. Correlation coefficients have been calculated connecting maltose figures with gas production (a) in 24 hours, (b) in the whole fermentation period, and (c) during final proof, with two different proportions of yeast. The results are given in Table II. No significant correlation was found between maltose figure and proving gas nor between maltose figure and total gas produced during the entire fermentation period (including final proof). This lack of correlation

occurs with long and with short fermentations, *e.g.*, 8 hours with $\frac{1}{2}\%$ yeast and $3\frac{1}{4}$ hours with 2% yeast. The only definite correlation found was between maltose figure and total gas produced in 24 hours—a correlation that is of little practical importance.³

TABLE II
CORRELATION COEFFICIENTS BETWEEN MALTOSE FIGURES AND GAS PRODUCTION

	Correlation coefficient	Probable error (P.E.) of the coefficient	P.E. $\times \frac{1}{6}$	Number of tests
Total gas production in 24 hours using $\frac{1}{2}\%$ yeast.....	0.695	± 0.040	.240	75
Total gas production in 8 hours using $\frac{1}{2}\%$ yeast.....	0.060	± 0.078	.468	71
Total gas production in $3\frac{1}{4}$ hours using 2% yeast.....	-0.273	± 0.072	.432	73
Gas production in 8th hour, using $\frac{1}{2}\%$ yeast.....	-0.023	± 0.082	.492	66
Gas production between $2\frac{1}{2}$ and $3\frac{1}{4}$ hours, using 2% yeast.....	0.281	± 0.070	.420	75

This lack of correlation is surprising in view of the known importance of maltose production in panary fermentation and especially when one remembers the ease with which gas production can be stimulated by the addition of malt extract to flour, *i.e.*, by increasing diastatic activity. The fact that the maltose test is carried out under conditions widely different from those that obtain in a dough may have a bearing on the problem. There is no *a priori* reason why maltose production should take place in a mixture of 30 g. of flour and 15 g. of water plus yeast and salt (*i.e.*, in a dough) at the same rate as in a mixture containing ten times as much water, and in the absence of yeast and salt. Yet this tacit assumption lies at the basis of the maltose test. Rumsey found that the maltose figure, as determined by his method, was independent of the amount of water used provided the water was at least equal in amount to the flour. If less than this minimum amount of water were present, as in a dough, the maltose figure might be reduced by as much as 20% (see Table III, section A). The Kent-Jones method of estimation is only approximate and its accuracy does not extend beyond the first decimal place. Determinations carried out by this method (see Table III, section B) in these laboratories were not conclusive, but do suggest a diminished

³ The high correlations found by Larmour, Geddes, and Whiteside (1933), Davis and Worley (1934) and by Graesser (1936) were between diastatic activity and *total* gas production using a very large excess of yeast. Such gas figures bear little relation to those obtained in fermenting doughs under ordinary commercial conditions. The object of the present investigation was to study the relationship of the maltose figure, as conventionally determined in England, to rate of gas production in ordinary panary fermentation with a view to seeing whether a test of diastatic activity can be used as a substitute for the cumbrous gassing test.

rate of maltose formation in dough as compared with more dilute flour-water mixtures. The effect, however, is small and is probably not a main factor in determining the absence of correlation between maltose figure and gas production.

TABLE III

EFFECT ON MALTOSE FIGURE OF VARYING THE WATER CONTENT OF THE FLOUR-WATER MIXTURE

A.—L. A. Rumsey—Using Rumsey's Method for Estimation of Maltose:—

Flour-water ratio	Maltose figure (%)
1 : 0.55	2.171
0.7	2.358
1.0	2.790
2.5	2.722
7.5	2.716
10.0	2.714
40.0	2.716

B.—E. A. Fisher—Using Kent-Jones' Approximate Method for Estimation of Maltose:—

Flour	Water	Flour-water ratio	Maltose figure	
	<i>C.c.</i>		<i>After 1 hour</i>	<i>After 7 hours</i>
30 g.	15	1 : 0.5	1.13	1.76
	30	1.0	1.41	2.46
	60	2.0	1.21	2.29
	85	2.83	1.19	2.31
	110	3.66	1.31	2.07
	130	4.33	1.18	2.36
	160	5.33	1.17	1.98

It has been established by several investigators (Rumsey, 1922; Collatz, 1922), and confirmed in the writers' laboratories, that in the absence of yeast reducing sugars will steadily accumulate in a dough. With yeast present maltose figure will increase for the first hour or so and will indeed usually exceed that obtained in the absence of yeast (owing to the production of glucose and fructose from cane sugar by yeast). After an hour or so, when the yeast has become fully active, maltose figure will decrease steadily throughout fermentation owing to the fact that the reducing sugars are utilized by the yeast faster than they are produced. The figures quoted in Table IV sufficiently establish this point.

It seemed possible that some light might be thrown on the problem by determining maltose figures at hourly intervals in the presence as well as in the absence of yeast. The results for two flours, *A* and *B*, both straight-run commercial flours, and for two different brands of yeast, are given in Table V. It is surprising that the yeast, instead of

TABLE IV

AMOUNTS OF REDUCING SUGARS (MALTOSE) CALCULATED AS PERCENTAGES OF DOUGH PRESENT IN DOUGH AT DIFFERENT STAGES OF FERMENTATION

Time	Percentage maltose		Time	Percentage maltose	
	No yeast	2½% yeast		No yeast	Approximately 3% yeast
	Rumsey			Collatz	
At mixing	1.257	1.560	At mixing	0.67	0.90
1 hour	1.594	2.095	1 hour	1.085	1.405
2 hr. 57 min.	2.041	2.055	2 hr. 30 min.	1.390	1.500
4 " 12 "	2.220	1.483	3 " 40 "	1.54	1.380
4 " 57 "	2.336	1.252	4 " 35 "	1.76	1.095
5 " 59 "	2.453	.846	5 " 8 "	1.745	1.015

Time	Percentage maltose
	2% yeast Fisher
At mixing	—
1 hour	1.545
2 hours	1.070
3 "	.300
4 "	Nil
5 "	"

reducing the maltose figure, as it does in a dough, actually increased it, the increase being progressive throughout the whole period of incubation. That is, under the conditions of the maltose test (large excess of water being present) yeast *increases* rate of production of reducing sugars, while in a dough yeast destroys these sugars as fast as or faster than they are produced.

To explore the problem further, gas-production figures and maltose figures were obtained at hourly intervals on a series of flour-water-yeast mixtures of varying water content. In Table VI are given gas-production figures for 30 g. of flour, 0.33 g. of yeast and varying amounts of water up to 160 c.c., the fermentation temperature being maintained at 26.7° C. In Figures 1 and 2 are given the hourly maltose figures for a similar series of mixtures only this time at the slightly different temperature of 27° C.

It will be noticed from Table VI that the rate of gas production is slightly greater when the flour and water concentrations are in the ratio 1 : 1 than when the concentrations are those in a dough, *viz.*,

2 : 1. When, however, the ratio of water to flour is further increased, the rate of gas production falls off progressively.

TABLE V

MALTOSE DETERMINATIONS AT HOURLY INTERVALS, WITH AND WITHOUT YEAST
Flour A

Time	Maltose figure	
	Without yeast	+ 1% D.C.L. yeast
1 hour	1.17	1.25
2 hours	1.33	1.47
3 "	1.48	1.69
4 "	1.66	1.94
5 "	1.80	2.24
5½ "	1.92	—
5¾ "	—	2.53
6½ "	1.90	2.57
7 "	1.98	2.81

Flour B

Time	Maltose figure		
	Without yeast	+ 1% D.C.L. yeast	+ 1% N.G. & S.F. yeast
1 hour	1.70	1.74	1.94
2 hours	1.83	1.93	2.39
3 "	2.00	2.16	2.81
4 "	2.24	2.41	3.17
5 "	2.40	2.69	3.50
5½ "	2.47	—	3.63
6 "	2.55	2.97	3.90
7 "	2.59	3.28	4.16

An examination of Figures 1 and 2 shows that the rate of maltose production changes in a characteristic manner with changing water content. With 70 c.c. of water per 30 g. of flour, the maltose content

TABLE VI

GAS PRODUCTION FROM 30 G. FLOUR + VARYING AMOUNTS OF WATER AND 0.3 G.
(= 1%) YEAST

Water in dough.....	15 c.c.	30	60	70	85	110	130	160
	C.c.			Gas figures				
1 hour.....	11	6	4	1	1	0	1	2
2 hours.....	30	19	10	6	4	4	3	4
3 ".....	56	50	29	25	17	12	11	11
4 ".....	92	95	66	59	55	38	31	38
5 ".....	140	150	120	105	83	65	51	55
6 ".....	192	210	168	151	127	89	67	65
7 ".....	238	253	198	181	164	109	85	73
8 ".....	264	276	220	205	176	123	97	79
24 ".....	438	492	452	444	412	233	139	95

of the suspension remained substantially the same from one to seven hours. With more water than this the maltose figure increased with increasing time, the rate of increase being greater the greater the content of water. On the other hand, with less water than 70 c.c. the maltose figure, although increasing during the first few hours, fell off rapidly with further increases in time. This fall-off was greater the lower the water content of the mixture.

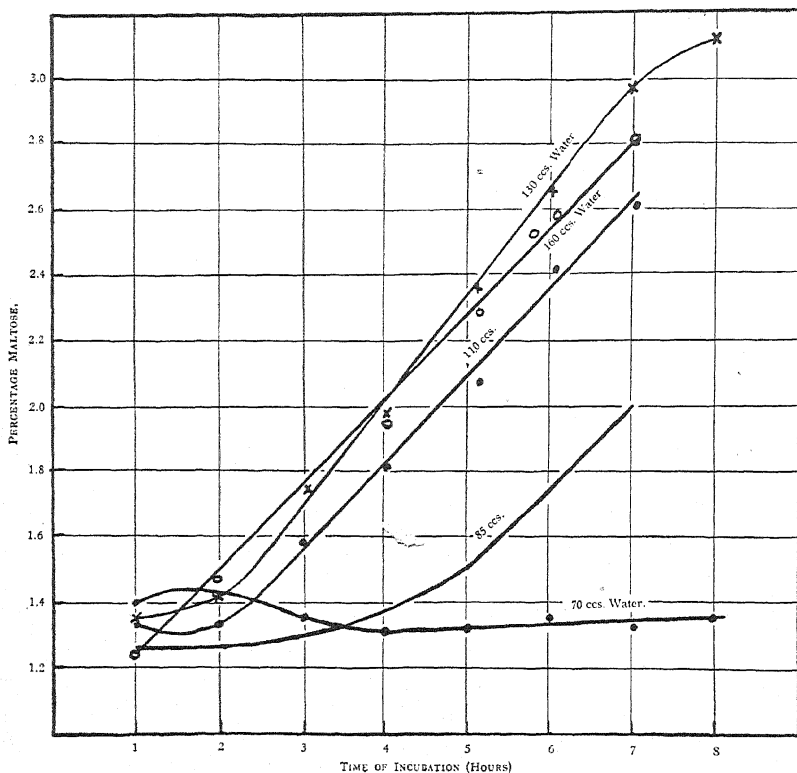
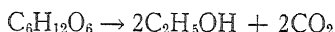


Figure 1. Hourly Maltose Production from 30 Grs. of Flour A + 1% D.C.L. Yeast and Varying Amounts of Water.

The reducing sugar content of a suspension at any given time is dependent on the balance between the sugar produced by the inversion of the original sucrose present and by the hydrolysis of the starch on the one hand, and the sugar used up by the yeast on the other hand. It is thus significant that the accumulation of maltose is greater in dilute suspensions where gas production is less, and is smaller where the flour concentration and therefore also gas production are greater.

To obtain an idea of the actual reducing sugar (expressed as mal-

tose) produced in these flour-water-yeast mixtures, the maltose consumed in the production of gas was calculated from the equation:



and on this basis Table VII was constructed. It is evident that the

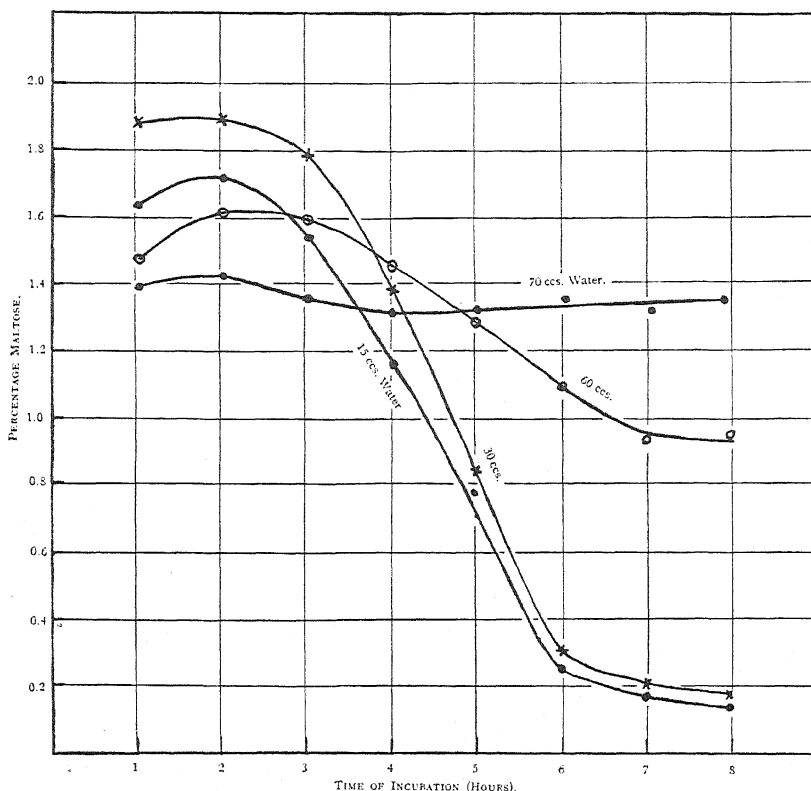


Figure 2. Hourly Maltose Production from 30 Grs. of Flour A + 1% D.C.L. Yeast and Varying Amounts of Water.

actual amount of maltose produced in any given suspension after any given time is equal to the sum of the maltose consumed in gas production and that remaining unused.

From some of the data in Table VII, Figure 3 was drawn. The curves in this figure corresponding to water contents of 15 and 30 c.c. per 30 g. of flour are apparently linear, and since with such low water concentration the inversion of the initial sucrose is almost complete within the first hour, it is probable that the slope of these curves gives a measure of the rate of maltose production through enzymic hydrolysis of starch. With more dilute mixtures the inversion of the sucrose

TABLE VII
MALTOSE FIGURES (REAL AND THEORETICAL) AT HOURLY INTERVALS OF FLOUR A
+ 1% D.C.L. YEAST

C.c. H ₂ O per 30 g. flour	15			30			60			70		
Hours	A ¹	B ²	C ³	A	B	C	A	B	C	A	B	C
1	.13	1.63	1.76	.07	1.87	1.94	.05	1.47	1.52	.01	1.39	1.40
2	.35	1.71	2.06	.22	1.89	2.11	.12	1.61	1.73	.07	1.42	1.49
3	.66	1.53	2.19	.59	1.78	2.37	.34	1.59	1.93	.29	1.35	1.64
4	1.08	1.18	2.26	1.12	1.34	2.46	.78	1.45	2.23	.70	1.31	2.01
5	1.65	.81	2.46	1.77	.95	2.72	1.42	1.32	2.74	1.24	1.32	2.56
6	2.27	.25	2.52	2.48	.29	2.77	1.98	1.09	3.07	1.78	1.34	3.12
7	2.81	.17	2.98	2.98	.20	3.18	2.34	.95	3.29	2.14	1.33	3.47
8	3.12	.14	3.26	3.26	.18	3.44	2.60	.94	3.54	2.42	1.35	3.77
24	5.17			5.20			5.32			5.23		
	85			110			130			160		
1	.01	1.26	1.27	0	1.33	1.33	.01	1.35	1.36	.02	1.25	1.27
2	.05	1.28	1.33	.05	1.33	1.38	.04	1.41	1.45	.05	1.47	1.52
3	.20	1.27	1.47	.14	1.53	1.67	.13	1.72	1.85	.13	1.69	1.82
4	.65	1.34	1.99	.45	1.76	2.21	.37	1.96	2.33	.45	1.94	2.39
5	.98	1.51	2.49	.76	2.00	2.76	.60	2.31	2.91	.65	2.24	2.89
6	1.50	1.72	3.22	1.02	2.39	3.41	.79	2.62	3.41	.77	2.53	3.30
7	1.93	1.99	3.92	1.29	2.37	3.66	1.01	2.96	3.97	.86	2.57	3.43
8	2.08	—	—	1.45	—	—	1.14	3.12	4.26	.93	2.81	3.74
24	4.86			2.76			1.64			1.06		

¹A = maltose consumed by yeast (calculated).

²B = maltose remaining in solution.

³C = total maltose produced (= A + B).

is more gradual (see Table V) and in consequence the maltose content of such mixtures is lower during the first few hours than in the case of the more concentrated mixtures.

It was shown in Table III that the production of maltose from starch in a flour-water mixture is almost independent of the flour concentration. If this were equally true for flour-water-yeast mixtures the curves in Figure 3 should, after sufficient time had been allowed for the complete inversion of the sucrose in each case, all reach the same maltose level. Since this is not the case, and the curves for the lower flour concentrations actually cross those for the higher flour concentrations reaching a much higher maltose level after 6 to 7 hours, it is apparent that the rate of production of maltose in flour-water-yeast mixtures is not independent of water content but increases with it.

The observations recorded in this paper are of a purely preliminary character. They have not solved the problem of the lack of correla-

tion between maltose figure and gas production during fermentation, but have exposed to view a number of other problems, important in their categories, for future investigation. The observations lend support to the view that the problems of panary fermentation are vastly more complex than may appear at first sight, and that the lack of correlation between maltose figure and gas-producing capacity is due to the wide differences in the conditions under which the maltose test is carried out and those obtaining in a dough.

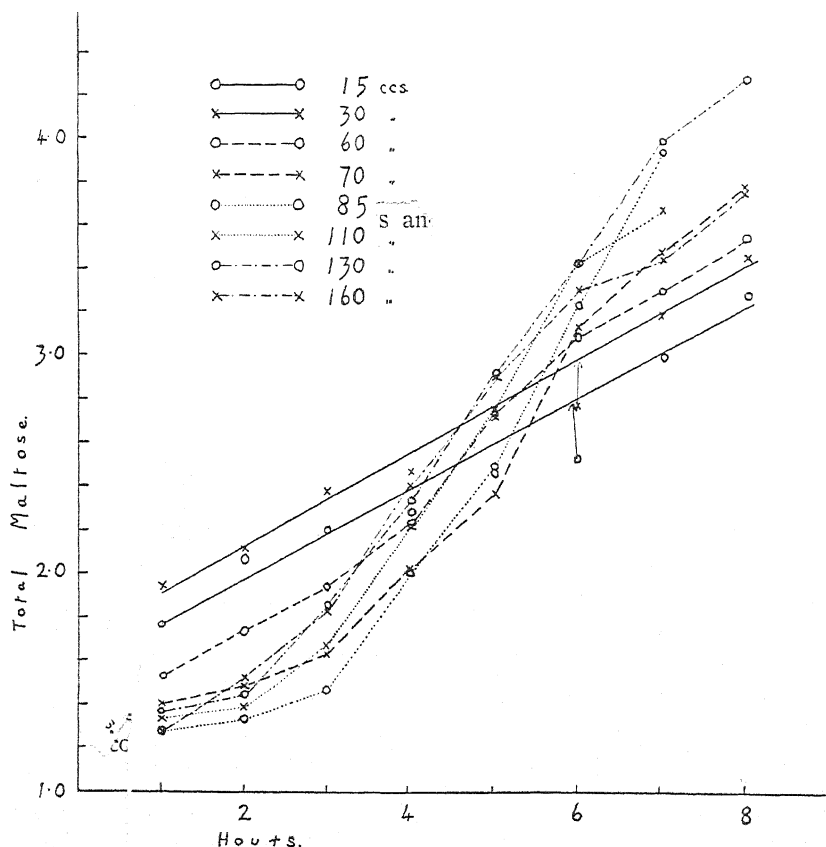


Figure 3.

It follows from this work that the maltose test cannot be used as a safe substitute for the gas test, and that a low maltose figure is not necessarily undesirable. The real importance of the maltose test lies at the other end of the scale: as shown in the writers' laboratories in 1926, it is flours of high maltose content, rather than low maltose flours, that are likely to give trouble in the bakehouse. Flours of

maltose figures higher than about 2.3 produce sticky doughs, the loaves refusing to bake or "soak."

Summary

The importance of diastatic activity of flour in its relation to adequate gas production during fermentation is emphasized. Diastatic action results in the formation in dough of maltose which is acted on by yeast with the production of alcohol and carbon dioxide. On this account some workers consider that rate of maltose production should afford a measure of the rate of gas production and have suggested that the familiar maltose test can be used as a substitute for the tedious and cumbersome gassing test. It is shown that there is no correlation at all between the maltose figure, as conventionally determined, and gas production either during final proof or throughout the whole course of fermentation, either short or long doughs. This complete absence of correlation between maltose figure and gas production is surprising and attempts have been made to explain it. The experimental work described has not completely solved the problem, but has indicated that the absence of correlation is due probably to the wide differences that exist between the conditions under which the maltose test is carried out and those obtaining in a dough. In the course of the work a number of fresh problems, important in their respective categories, have been exposed for future investigation.

As a result of this work it is emphasized that the routine maltose test cannot be used as a safe substitute for the gas test. The real value of the maltose test is due to the dangers inherent in flours of high maltose content.

Literature Cited

- Blish, M. J., Sandstedt, R. M., and Astleford, G. R.
1932 Sugars, diastatic activity, and "gassing power" in flour. *Cereal Chem.* 9: 378-393.
- Collatz, F. A.
1922 Flour strength as influenced by the addition of diastatic ferment. *Amer. Inst. Baking, Bull.* 9.
- Davis, C. F., and Worley, D. F.
1934 Correlation between diastatic activity and gassing power in commercial flours. *Cereal Chem.* 11: 536-545.
- Graesser, F. R.
1936 Correlations between loaf volume, crumb characters, baking value, and diastatic activity in Canadian western hard wheat flours. *Cereal Chem.* 13: 356-363.
- Humphries, A. E.
1907 Reports of the Home Grown Wheat Committee of the National Association of British and Irish Millers (London).
- Jørgensen, H.
1931 The separation of "gassing power" (diastatic activity) from "strength" in baking tests. *Cereal Chem.* 8: 361-374.
- Kent-Jones, D. W.
1924 *Modern Cereal Chemistry* (London), First Edition, pp. 255-261.

- Landis, O., and Frey, C. N.
1936 The enzymes of flour in relation to yeast fermentation. *Cereal Chem.* 13: 281-317.
- Larmour, R. K., Geddes, W. F., and Whiteside, A. G. O.
1933 Comparison of various baking formulas used in testing wheat quality for the plant breeder. *Cereal Chem.* 10: 601-604.
- Rumsey, L. A.
1922 The diastatic enzymes of wheat flour and their relation to flour strength. *Amer. Inst. Baking, Bull.* 8.
- Steller, W. R., Markley, M. C., and Bailey, C. H.
1935 Factors affecting the diastatic activity of wheat flour. II. *Cereal Chem.* 12: 268-275.
- Wood, T. B.
1907 The chemistry of strength of wheat flour. Part I. The size of the loaf. *J. Agr. Sci.* 2: 139-160.

THE ISOLATION OF THE COMPOUND GIVING YELLOW CORN ITS CHARACTERISTIC ODOR¹

I. B. JOHNS and W. OLDHAM

Department of Chemistry, Iowa State College, Ames, Iowa

(Received for publication January 21, 1938)

In connection with another problem, it has been observed that saponification of fresh yellow corn oil does not destroy its characteristic odor. The non-saponifiable matter contains, among other things, sterols, the pigments, and the odoriferous substances. The study of these latter compounds has been initiated with the aim of first finding a convenient method of isolation from whole corn, second, localizing them in the corn kernel, and third, determining their chemical structures.

The substances seem to be present in the oil and to give to it the characteristic odor whether it is obtained by pressing or extracting. Likewise, corn from which all the oil has been extracted no longer possesses the odor. This would lead one to conclude that the odoriferous substances may be a part of the oil. Furthermore, the parts of the corn which contain little or no oil possess little or no odor. Although the odor can be removed from the oil by steam distillation and appears apparently unchanged in the condensate, it cannot be obtained from whole corn by this method.

The odoriferous material can be obtained by direct steam distillation of the oil or of the non-saponifiable part of the oil. The former appears to be the best, since the saponification of large amounts of oil and especially the working up of the resulting soaps is an arduous task. Accordingly, a trial run was made using 375 grams of oil that had been extracted from ground yellow corn by means of ethyl ether. This oil

¹ Journal Paper No. J-530 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 567.

was steam-distilled until no appreciable odor was left. The distillate, 1500 c.c., possessed a pungent odor very suggestive of yellow corn. It also contained some free fatty acids and so was made alkaline with sodium hydroxide and steam-distilled again. During this distillation the odorous substances passed over much more rapidly than from the original oil. By repeated partial distillation of each condensate, the material was concentrated into a final condensate of 40 c.c. From this solution the odorous substance was removed with ether. It was then dried over solid KOH, decanted, and evaporated off. The residue was a very small quantity of a light yellow oil. This oil was distilled up to 220° C., yielding 6 milligrams of a nearly colorless liquid and leaving a brown waxy residue. Although the distillate was definitely a mixture, it was analyzed and found to contain 76.77% C and 11.65% H. It contained no N nor S. Its molecular weight, using bornyl bromide as the cryoscopic medium, was found to be 260. The substance possessed a powerful odor, the slightest trace of it on a glass thread being noticeable throughout the room.

A larger run was made using 10 liters of pressed oil. After the above process of concentration and extraction, 117 milligrams of crude material were obtained. This was fractionally distilled at reduced pressures and yielded two distinct fractions. The first, 6 milligrams distilling at 150°–152° C. and 21 mm., was a clear liquid. Analysis showed it to have a molecular weight of 156, to contain one oxygen atom and to have a carbon-hydrogen ratio of 1:2. The second fraction, 42 milligrams distilling at 190°–200° C. at 3.5 mm., was a waxy solid melting slightly above room temperature. Analysis showed it to have a carbon-hydrogen ratio of 1:2, to contain only 2.5% oxygen, and to have a molecular weight of 464. Since the complete separation of such small quantities of a mixture by a single fractional distillation is not possible, it is certain that each fraction was contaminated with some of the other. Accordingly, the high boiling substance may be a hydrocarbon and the low boiling substance an oxygen containing compound of still lower molecular weight. With the material at hand it was impossible to further separate these mixtures by repeated fractionation. The second fraction did not appear to be the odoriferous compound sought as small amounts, on exposure to air, soon lost all traces of the odor, apparently held temporarily by absorption. The first fraction possessed a permanently strong odor.

The yield from such a large amount of pressed oil was much lower than might have been expected from the results of the trial run using extracted oil. One must therefore conclude that the oil extracted by means of ether contains much more of the odorous compound. To

establish this, 600 grams each of pressed oil, extracted oil from whole corn, and extracted oil from yellow corn germ, were steam-distilled until 1 liter of condensate was collected from each portion and the odors then qualitatively compared. The condensates from the two portions of extracted oil were apparently equal in pungency while that from the pressed oil possessed much less odor. There are two possible explanations for this. The odorizing material may be held in the germ in such a manner that the pressing out of the oil does not carry the odorizer with it. On the other hand the temperature at which the pressing process is carried out may be sufficient to distill out a portion of the odoriferous material. At any rate, the amount of the compound present in yellow corn appears to be surprisingly low. Extractions were carried out on white corn but the resulting oils possessed so little odor that no attempt was made to isolate the odorous compounds.

The authors wish to express their appreciation of the kindness of the Miller Cereal Mills of Omaha in supplying them with materials.

Summary

A volatile compound having the characteristic odor of yellow corn has been isolated from yellow corn oil.

It is a compound with a molecular weight of less than 150 and contains one atom of oxygen.

The yield was approximately one part per million parts of pressed oil.

A COMPARISON BETWEEN THE STANDARD BASIC AND MALT-PHOSPHATE-BROMATE BAKING METHODS ON 1937 NORTH DAKOTA HARD RED SPRING WHEAT

R. H. HARRIS and T. SANDERSON

North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Received for publication November 29, 1937)

Introduction

The problem of evaluating the inherent quality of wheat flour has been widely discussed by cereal chemists, and a variety of baking methods has been suggested for accomplishing this purpose. One flour may vary so widely in its response to different baking procedures that it becomes increasingly difficult for the technologist to place properly the flour in regard to baking quality. It is probable that the term "potential baking quality" would be of more general utility as suggested by Cayzer (1936), as it is an absolute expression, and may therefore be used with less reserve. As used by Cayzer, this term signifies the ability of a flour to yield satisfactory baking results under severe conditions of baking procedure. The higher the "potential baking quality" of a flour the larger the loaf produced as these conditions are made more drastic. It must be borne in mind, however, that the imposition of too severe conditions will reduce the loaf volume of high protein flours to a greater degree than those of lower protein content, as postulated by Aitken and Geddes (1934).

Geddes and Larmour (1933) concluded that the inclusion of potassium bromate in the baking formula led to a better differentiation of baking quality in Western Canadian hard red spring wheat flours. The correlation of loaf volume with protein content was higher with the bromate than when the basic formula was used, and the bromate method was more sensitive in bringing out modifications in baking quality. The basic method was influenced more by variation in the enzymic activity of the flour. In the higher protein ranges larger increments of bromate would probably be required to bring out the full potentiality of a flour. Larmour, Geddes, and Whiteside (1933) found the malt-phosphate-bromate formula to give the greatest range of loaf volume when testing various hybrids and standard varieties for Canadian plant breeders. The effect of diastatic activity appeared to be ruled out by this baking procedure. The authors did not con-

clude that the malt-phosphate-bromate formula was superior to the bromate formula for the type of work cited.

Blish (1934) cited the utility of the basic procedure of the Standard A. A. C. C. baking test as a point of reference. Other and supplementary procedures could be used to obtain additional information regarding flour quality.

Aitken and Geddes (*loc. cit.*) found that the malt-phosphate-bromate formula with a 3-hour fermentation period yielded the largest absolute loaf volumes, as well as maximum differentiation, when 10 composite wheat samples obtained by blending the surpluses of samples from the 1932 protein survey of Western Canadian hard red spring wheat were baked by a variety of methods. By imposing severe baking conditions the loaf volume of the higher protein flours was reduced to a greater degree than those of lower protein content, thus decreasing the differences between the flours. Loaf volume alone was considered to be an index of baking quality in this investigation.

In a study of various methods of evaluating baking quality, Kent-Jones and Geddes (1936) employed a number of formulas, including the basic, malt-phosphate, malt-phosphate-bromate, and malt-phosphate-bromate with variable mixing time. A wide range of baking quality existed in the series of flours used, and it was found that the malt-phosphate-bromate formula with 3-minute mixing time in a Hobart-Swanson mixer yielded maximum differentiation among the flours. The malt-phosphate-bromate formula with the usual (1-minute) mixing time ranked second in differentiating the different samples.

The application of the bromate supplement to North Dakota hard red spring wheat has not always appeared to yield satisfactory results in the form of increased loaf volume and better differentiation when compared with the standard basic method. This fact has not been in accord with the author's experience in working with hard red spring wheats grown in Western Canada, or with several samples previously tested of American grown wheat. It did not seem probable that a difference of a few miles in distance would cause such marked differences in the environment as would alter to any appreciable extent a fundamental attribute of wheat such as positive response to a properly balanced baking formula. In view of these considerations it was considered desirable to study the effect of the malt-phosphate-bromate formula in comparison with the standard basic procedure upon the current crop of North Dakota hard red spring wheat.

Experimental

MATERIAL AND METHODS

A series of 89 samples of hard red spring wheat grown in the State of North Dakota during the crop season of 1937 were experimentally

TABLE I

COMPARATIVE GRADES AND ANALYTICAL DATA OF SAMPLES ARRANGED IN ORDER OF INCREASING WHEAT PROTEIN CONTENT¹

Laboratory number	Grade ²	Crude protein (N × 5.7)		Moisture	Ash	Diastatic activity mg. maltose per 10 g. flour
		Wheat	Flour			
		%	%	%	%	
37-8-31	4 DNS	11.1	10.3	14.0	0.50	146.0
44	1 DNS	11.4	10.7	14.2	.46	140.7
27	4 DNS	12.1	11.3	14.7	.46	131.8
25	3 DNS	12.2	11.6	13.9	.48	158.2
35	4 DNS	12.3	11.8	14.7	.54	154.4
37-9-38	S DNS	12.3	11.6	14.3	.52	106.0
37-8-15	1 NS	12.4	11.5	14.1	.47	119.4
36	1 DNS	12.4	11.9	14.5	.47	129.0
37-9-40	S DNS	12.5	12.1	13.5	.57	167.0
37-8-33	S DNS	12.9	12.1	14.6	.58	126.5
43	5 DNS	12.9	12.0	14.2	.49	101.5
37-9-30	2 DNS	12.9	12.4	14.3	.48	151.4
29	3 DNS	13.0	12.5	14.2	.48	167.1
33	3 DNS	13.0	12.4	14.0	.50	158.6
37-8-34	1 DNS	13.2	12.1	14.9	.51	126.6
37-9-2	2 DNS	13.2	12.7	14.4	.47	121.4
15	S DNS	13.2	12.1	13.5	.61	177.0
37-8-5	1 HvRS	13.3	12.2	13.9	.45	137.0
38	1 DNS	13.4	12.9	14.3	.46	123.5
37-9-14	S DNS	13.5	12.6	13.5	.63	174.2
37-8-20	2 DNS	13.6	13.2	13.6	.46	135.5
51	S DNS	13.6	12.3	12.3	.51	106.0
37-9-21	4 DNS	13.6	12.7	14.4	.50	150.0
37-8-42	5 DNS	13.8	13.3	14.3	.41	105.0
37-9-4	S DNS	13.8	13.2	14.2	.56	123.5
20	1 DNS	13.8	13.2	13.6	.46	135.5
22	S DNS	13.9	12.9	13.9	.62	136.0
31	S DNS	13.9	13.3	13.9	.50	123.7
37-8-26	4 DNS	14.0	13.3	13.8	.44	144.4
37-9-5	1 HvNS	14.0	13.7	14.6	.45	91.2
32	S DNS	14.0	13.2	14.2	.55	145.4
37-8-8	S DNS	14.1	13.0	14.2	.55	146.0
50	5 DNS	14.1	13.8	13.0	.51	97.0
47	S DNS	14.2	13.1	14.1	.54	148.0
37-9-6	S DNS	14.2	13.8	14.7	.51	114.8
34	S DNS	14.2	13.3	14.1	.47	106.5
1	S DNS	14.3	13.2	14.0	.58	120.4
28	3 DNS	14.3	13.7	14.1	.49	121.0
37-8-11	3 DNS	14.4	13.9	14.7	.49	129.0
21	5 DNS	14.4	13.8	14.4	.65	137.0
37	S DNS	14.4	13.8	14.8	.59	99.0
37-9-12	S NS	14.4	13.4	13.4	.60	147.3
16	4 DNS	14.4	14.0	14.6	.53	99.4
24	5 DNS	14.4	13.2	14.4	.72	100.0
37-9-25	4 DNS	14.4	13.7	14.8	.47	98.0
37-8-9	3 DNS	14.5	13.9	13.4	.52	158.2
30	2 DNS	14.5	13.8	13.3	.56	162.0

TABLE I—*Continued*

Laboratory number	Grade ²	Crude protein (N × 5.7)		Moisture	Ash	Diastatic activity mg. maltose per 10 g. flour
		Wheat	Flour			
		%	%	%	%	
37-9-18	4 DNS	14.5	13.6	14.8	0.46	98.7
26	S DNS	14.5	13.6	14.5	.54	114.5
37-8- 4	5 NS	14.6	14.0	14.3	.46	88.7
18	4 NS	14.6	14.2	14.5	.52	118.0
28	S DNS	14.6	13.3	13.8	.57	140.7
37-9-10	S NS	14.6	14.0	14.1	.49	113.4
23	3 DNS	14.6	13.8	13.9	.50	138.0
37	S DNS	14.6	13.8	14.1	.49	110.8
37-8-45	4 DNS	14.7	14.2	14.6	.41	103.3
48	3 DNS	14.8	14.1	13.7	.48	142.0
37-9-36	S NS	14.8	13.8	13.4	.52	126.0
37-8-41	5 DNS	14.9	14.1	14.2	.54	133.2
46	2 DNS	14.9	14.6	14.3	.47	110.0
29	4 DNS	15.0	14.4	14.4	.46	102.4
12	5 DNS	15.1	13.8	14.2	.56	131.0
37-9-11	S NS	15.1	14.4	14.0	.62	142.0
35	S DNS	15.1	14.5	13.4	.50	122.5
37-8- 1	5 NS	15.2	14.1	15.2	.50	82.0
37-9- 9	S DNS	15.2	14.6	14.1	.54	94.0
37-8-14	1 DNS	15.4	14.6	13.9	.43	125.5
22	3 DNS	15.4	14.9	14.2	.65	164.5
37-9-27	S DNS	15.4	14.4	13.9	.58	125.5
37-8-19	S NS	15.5	14.7	13.9	.59	113.5
40	S DNS	15.5	14.8	14.3	.56	121.8
37-9- 3	S DNS	15.6	15.2	14.2	.49	106.4
37-8- 39	3 DNS	15.6	15.0	13.6	.53	153.0
37-8- 7	5 NS	15.7	15.0	14.0	.48	83.7
3	4 NS	15.8	15.0	14.3	.44	88.9
2	S NS	16.0	15.4	14.1	.55	115.6
32	4 DNS	16.0	15.4	13.8	.59	125.4
37-9- 8	3 DNS	16.1	15.7	14.2	.50	100.8
37-8-13	5 DNS	16.3	15.4	13.6	.52	104.9
37-9- 7	1 DNS	16.3	15.4	14.5	.51	102.0
37-8-23	5 DNS	16.4	16.3	13.8	.72	165.6
37-8-39	5 DNS	16.5	16.2	14.1	.49	80.0
37-8-24	5 DNS	17.0	15.6	13.4	.67	224.7
17	S NS	17.1	16.2	13.3	.95	132.4
37-9-13	S NS	17.5	16.0	13.1	.76	210.3
37-8-49	5 DNS	17.7	17.2	13.8	.49	85.7
6	S NS	18.0	17.0	14.0	.44	76.0
10	S DNS	19.0	17.6	14.5	.51	74.8
16	S NS	19.3	19.1	14.0	.49	102.6

¹ Analytical results calculated to 13.5% moisture basis.² Unofficial.

milled into straight flour on an Allis-Chalmers mill. These wheats were all free from damage by bleaching or weathering but some had been affected by rust and heat. An effort was made to secure an even distribution of the samples from the different counties in the State, but owing to drought conditions in the Western portion no samples

TABLE II

COMPARATIVE RESULTS OBTAINED BY THE TWO BAKING METHODS ARRANGED IN ORDER OF INCREASING WHEAT PROTEIN CONTENT

Laboratory number	Ab-sorption	Baking procedure					
		Standard			Malt-phosphate-bromate ¹		
		Loaf volume	Color	Texture	Loaf volume	Color	Texture
		<i>c.c.</i>			<i>c.c.</i>		
37-8-31	60	476	94	95	556	94	94
44	60	495	94	95	581	94	93.5
27	61	505	94	93	570	95	94
25	60	500	92	93	655	95	93
35	61	510	92	93	641	93	93
37-9-38	64	533	94	94	580	93	92
37-8-15	59	511	92	94	550	94	94
36	62	502	93	93	612	94	93
37-9-40	65	529	92	94	661	91	92
37-8-33	60	553	92	93	698	93	92
43	61	522	92	95	660	93	93
37-9-30	64	544	93	92.5	630	94	91
29	63	551	94	93	636	93	91
33	60	545	92	92	694	94	92
37-8-34	62	502	93	93	575	93	92
37-9-2	62	515	94	94	675	94	91
15	65	545	92	92	652	93	93
37-8-5	61	546	93	94	588	94	94
38	60	542	93	93	653	94	93
37-9-14	64	545	92	94	698	94	93
37-8-20	60	533	92	94	711	95	93
51	63	560	92	92	712	93	93
37-9-21	63	591	95	94	662	93	93
37-8-42	61	544	94	94	650	94	93
37-9-4	62	568	92	94	665	92	93
20	62	577	94	94	710	94	92
22	63	555	93	92	700	93	91
31	63	556	92	94	762	93	91
37-8-26	60	542	94	95	655	95	94
37-9-5	60	515	94	94	677	95	93
32	62	573	93	94	675	93	92
37-8-8	61	584	92	94	631	93	93
50	63	556	92	94	752	93	92
47	63	565	92	92	720	93	93
37-9-6	61	596	94	92	669	92	91
34	63	554	94	92	724	94	92
1	62	528	93	94	625	94	93
28	61	548	94	92	735	94	91.5
37-8-11	62	564	92	94	695	94	90.5
21	60	588	93	92	706	94	94
37	60	596	92	92	662	92	93
37-9-12	63	543	94	93.5	737	94	93
16	60	530	92	92	716	93	92
24	65	548	92	92	700	94	91

TABLE II—*Continued*

Laboratory number	Ab-sorption	Baking procedure					
		Standard			Malt-phosphate-bromate ¹		
		Loaf volume	Color	Texture	Loaf volume	Color	Texture
		<i>c.c.</i>			<i>c.c.</i>		
37-9-25	61	545	93	94	743	94	90.5
37-8- 9	62	553	90	94	707	94	92
30	62	582	94	92	664	93	94
37-9-18	62	532	93	92	795	93	92
26	63	549	93	94	743	94	92
37-8- 4	60	540	92	94.5	725	93	92
18	60	572	93	92	730	95	92
28	62	634	93	92	677	92	94
37-9-10	62	539	93	93.5	700	94	93
23	63	557	94	94	817	94	91
37	64	569	94	92	737	92	91
37-8-45	62	511	92	94.5	745	93	91
48	63	582	92	94	745	94	93
37-9-36	63	563	93	92	735	92	91
37-8-41	61	526	94	94	668	94	93
46	62	560	94	94	768	93	91
29	62	537	94	95	778	93	92
12	61	608	90	94	743	92	91
37-9-11	62	592	92	94	810	94	93
35	63	601	95	92	860	92	91
37-8- 1	59	548	92	92	658	91	91.5
37-9- 9	63	548	92	92	758	93	91
37-8-14	62	598	94	93.5	730	94	92
22	60	616	93	92	800	93	93
37-9-27	62	604	91	93	820	92	92.5
37-8-19	62	528	88	94	703	92	92
40	60	578	92	92	672	92	91
37-9- 3	62	545	94	92	830	95	93
39	65	629	95	94	780	92	91
37-8- 7	60	521	93	93	768	95	92
3	60	550	93	94	852	95	90
2	60	578	90	95	690	92	92
32	61	557	94	92	758	93	92
37-9- 8	62	591	94	92	818	93	90
37-8-13	61	600	93	92	715	93	92
37-9- 7	61	561	94	93	788	93	90
37-8-23	60	550	91	93	758	93	90.5
37-8-39	61	562	92	92	840	93	92.5
37-8-24	61	632	91	92	825	93	90
17	60	645	84	93	880	90	91
37-9-13	62	622	91	92	817	92	92.5
37-8-49	63	562	91	92	825	93	91
6	60	556	93	93	933	93	90
10	60	563	90	93	793	93	92
16	60	661	93	92	1070	93	92

¹ 0.3% 60° Lintner malt, 0.1% ammonium phosphate and 0.001% KBrO₃.

were obtained from several counties. The samples were cleaned from dockage and scoured before milling, then tempered to a moisture content of 15% for 20 hours prior to milling.

The protein content of the wheat and flour was determined, also flour ash, moisture, and diastatic activity. The flours were then baked by two methods—the standard basic and the malt-phosphate-bromate formulas.¹ Both formulas had 5% of sucrose included instead of the usual 3%.

THE DATA

The wheat grades, crude wheat and flour protein, ash, moisture and diastatic activity are shown in Table I. The items are arranged in order of increasing wheat protein content, as this variable is commonly considered to be an index of baking quality. A scrutiny of the data shows that wheat and flour protein are very closely associated, the flour protein running lower than the corresponding wheat protein due to the removal in the milling process of the branny layer with its attendant high protein content. A large variability in wheat grades is evident, reflecting the variation in the environment and rust incidence among the samples. The ash is somewhat variable but does not appear to be closely associated with the grade among the different flours.

The baking results obtained, in terms of loaf volume, color, and texture, are shown in Table II, arranged as before in order of increasing crude wheat protein content. When loaf volumes obtained by the two baking methods are compared, an increase in loaf volume with the malt-phosphate-bromate method is noticeable in every instance. This positive response varies from 39 to 409 c.c., and is especially evident among the higher protein flours. The color of the loaves was increased by the malt-phosphate-bromate formula, but the texture was coarser owing principally to increased size of the loaf with consequent gas pockets in the interior. The flour milled from the lower grades appeared to average the same in absorption as the flour from the better grades. While the color of the crumb does not appear to change materially with increase in wheat protein, the texture score of the loaves baked by the malt-phosphate-bromate method tends to decrease with increasing protein. This is caused by the greater expansion produced by this formula with high protein flour.

In Table III are shown the responses to the malt-phosphate-bromate formula in cubic centimeters of difference between the loaf volume obtained by the two methods. As the responses were all positive, no algebraical sign was included in the table. A perusal of

¹ 0.3% 60° Lintner malt, 0.1% ammonium phosphate and 0.001% KBrO₃.

the data reveals increased response as the protein content increases. This again would be expected because of the larger loaves obtained by the use of the malt-phosphate-bromate formula. This response to an improver has been suggested as a criterion of flour strength because it measures the increase in loaf size produced under the stimulus of the improver. Here the standard basic loaf volume is used as a point of reference, as proposed by Blish (*loc. cit.*).

TABLE III
RESPONSE TO MALT-PHOSPHATE-BROMATE FORMULA

Laboratory number	C.c.	Laboratory number	C.c.	Laboratory number	C.c.
37-8-31	80	37-9-32	102	37-8-29	241
44	86	37-8- 8	47	12	135
27	65	50	196	37-9-11	218
25	155	47	155	35	259
35	131	37-9- 6	73	37-8- 1	110
37-9-38	47	34	170	37-9- 9	210
37-8-15	39	1	97	37-8-14	132
36	110	28	187	22	184
37-9-40	132	37-8-11	131	37-9-27	216
37-8-33	145	21	118	37-8-19	175
43	138	37	66	40	94
37-9-30	86	37-9-12	194	37-9- 3	285
29	85	16	186	39	151
33	149	24	152	37-8- 7	247
37-8-34	73	25	198	3	302
37-9- 2	160	37-8- 9	154	2	112
15	107	30	82	32	201
37-8- 5	42	37-9-18	263	37-9- 8	227
38	111	26	194	37-8-13	115
37-9-14	153	37-8- 4	185	37-9- 7	227
37-8-20	178	18	158	37-8-23	208
51	152	28	43	37-8-39	278
37-9-21	71	37-9-10	161	37-8-24	193
37-8-42	106	23	260	17	235
37-9- 4	97	37	168	37-9-13	195
20	133	45	234	37-8-49	263
22	145	37-8-48	163	6	377
31	206	37-9-36	172	10	230
37-8-26	113	37-8-41	142	16	409
37-9- 5	162	46	208		

The correlation coefficients and other statistical constants are grouped in Table IV. The mean protein content between wheat and flour shows a difference of only 0.74% for all the samples included in this study. The flour protein tends to be slightly more variable than the wheat protein and this can probably be explained by small variations in the milling technique. The malt-phosphate-bromate (B) baking method produced loaves which averaged 161 c.c. higher than the standard basic (A) method. The malt-phosphate-bromate method also gave better differentiation between the samples. All the corre-

lation constants tabulated are very significant. Flour protein could evidently be predicted from the knowledge of wheat protein in material of this nature. The relationship between these variables is not entirely constant, however, as pointed out by Waldron and Mangels (1932). These workers found the lowest correlation constant between these characters to exist in the instance of nursery samples where diverse strains or varieties are included. Flour protein and loaf volume were more highly related when baking method (B) was employed.

TABLE IV
TABLE OF STATISTICAL CONSTANTS
Means, Standard Deviations and Coefficients of Variability

	Means	Standard deviation	Coefficient of variability
Wheat protein	14.55	1.51	10.38
Flour protein	13.81	1.51	10.93
Ash	0.526	0.083	15.78
Diastatic activity	125.57	22.824	18.18
Loaf volume (A)	557.77	35.04	6.28
Loaf volume (B)	718.95	84.99	11.82

SIMPLE CORRELATION COEFFICIENTS

Variables correlated			
X	Y	r_{xy}	P ¹
Flour protein	Loaf volume (A) ²	+ .5892	< .0001
Flour protein	Loaf volume (B) ³	+ .8361	< .0001
Flour protein	Wheat protein	+ .9818	< .0001
Loaf volume (A) ²	Loaf volume (B) ³	+ .5733	< .0001

¹ P = probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.

² A = Standard baking method.

³ B = Malt-phosphate-bromate method.

The significance of the differences between the correlation coefficients computed between flour protein and the loaf volume data is shown in Table V. Fisher's (1928) method of transformation of the correlation coefficient to the Z value was used. The significance of the difference may be computed by finding the relative deviate X^1 from the ratio of the difference in Z value ($Z_1 - Z_2$) to its standard deviation $\left(\sqrt{\frac{1}{N_1 - 3} + \frac{1}{N_2 - 3}} \right)$, N_1 and N_2 in the present instance being 89. From a knowledge of the relative deviate the probability of the observed difference occurring owing to the forces of chance incidence may be evaluated. It is apparent that there is a significant

difference between the correlation coefficients obtained between flour protein and loaf volumes yielded by the two baking methods.

TABLE V

TESTS OF SIGNIFICANCE OF DIFFERENCE BETWEEN CORRELATION COEFFICIENTS
COMPUTED FROM FLOUR PROTEIN AND LOAF VOLUME DATA

Variable Correlated with Flour Protein	Correlation	Zr	$d/\sigma d$	X^1	P
Formula (B)	.8361	1.2083	.5320		
Formula (A)	.5892	0.6763	$\sqrt{1/43}$		
difference (d)		0.5320		3.488	.0005

Discussion of Results

The malt-phosphate-bromate formula with 2-minute mixing time in the Hobart-Swanson gave very satisfactory results with the samples tested from the 1937 crop of North Dakota hard red spring wheat. Higher loaf volumes were registered by its use, and the crumb color was improved when compared with the corresponding loaves baked by the standard basic method. These results are characteristic of wheats possessing high inherent quality and such wheats and the flours milled therefrom should accordingly be suitable for blending with weaker and "softer" wheats to improve the "potential baking quality" of the blends.

From the data included in this investigation it would seem that the standard basic method should be used in conjunction with a second method, or possibly more methods, which would tend to bring out the inherent possibility of a flour, or the "potential baking quality," using the term suggested by Cayzer (*loc. cit.*).

In a study of this nature flour protein could be estimated with sufficient accuracy for practical purposes from a knowledge of wheat protein. Similarly, wheat protein could be estimated from a knowledge of flour protein. The statistical constants are shown in Table VI.

TABLE VI

LINEAR REGRESSION COEFFICIENTS	
Flour protein % ^(w) on wheat protein % ^(x) , $b =$	.9827
Wheat protein % ^(x) on flour protein % ^(w) , $b^1 =$	.9809
LINEAR REGRESSION EQUATIONS	
Estimation of flour protein % ^(w) from wheat protein % ^(x)	
$Y = - .4827 + .9827x$	
Error of estimate =	.29%
Estimation of wheat protein % ^(x) from flour protein % ^(w)	
$X = + .9983 + .9809y$	
Error of estimate =	.29%

Summary and Conclusions

A straight flour was milled from 89 samples of North Dakota hard red spring wheat of the 1937 crop. Analytical determinations were run upon the wheat and flour, and the flours were baked by two methods—the standard basic with 5% sucrose, and the malt-phosphate-bromate. Response in cubic centimeters to the latter formula was calculated.

An examination of the data revealed an extremely high positive correlation between wheat and flour protein. This relationship could be used for predicting one variable from a knowledge of the other. The loaf volumes obtained by both methods of baking were significantly and positively correlated with the flour protein.

It was shown that the malt-phosphate-bromate method gave a significantly higher correlation with flour protein than the standard basic procedure.

North Dakota hard red spring wheat flour was shown to respond to the malt-phosphate-bromate formula in a satisfactory manner, yielding larger loaves of improved color, and giving better differentiation among the various samples. The higher protein flours gave loaves of coarser texture than the low protein samples.

Literature Cited

- Aitken, T. R., and Geddes, W. F.
1934 The behaviour of strong flours of widely varying protein content when subjected to normal and severe baking procedures. *Cereal Chem.* 11: 487-504.
- Blish, M. J.
1934 The laboratory baking test—a science or a fine art? *Cereal Chem.* 11: 70-73.
- Cayzer, Leonard S.
1936 Strength or baking quality of wheat. Its determination by baking tests. *J. Aus. Inst. Agr. Sc.* 2: 57-62.
- Fisher, R. A.
1928 Statistical methods for research workers. Second edition, p. 170. Oliver and Boyd, London.
- Geddes, W. F., and Larmour, R. K.
1933 Some aspects of the bromate baking test. *Cereal Chem.* 10: 30-72.
- Kent-Jones, D. W., and Geddes, W. F.
1936 A co-operative study of the utility of different methods for evaluating flour strength. *Cereal Chem.* 13: 239-280.
- Larmour, R. K., Geddes, W. F., and Whiteside, A. G. O.
1933 Comparison of various baking formulas used in testing wheat quality for the plant-breeder. *Cereal Chem.* 10: 601-604.
- Waldron, L. R., and Mangels, C. E.
1932 Correlational and allied studies of the protein content, water absorption, loaf volume, and loaf weight of two series of hard red spring wheats. *J. Agr. Research* 45: 209-231.

THE RELATION OF FLOUR STRENGTH, SOY FLOUR AND TEMPERATURE OF STORAGE TO THE STALING OF BREAD¹

W. R. STELLER² and C. H. BAILEY

(Read at the Annual Meeting, May 1937)

As wheat flour bread ages several distinct changes in its properties take place. These commonly include loss of water, loss of flavor and aroma, crust staling and crumb staling. The changes which have been found to occur in crumb staling include the lowering of the absorptive power of the starch, the decrease in the content of water-soluble amylose, the hardening of the crumb, the development of crumbliness and the accompanying change in the X-ray pattern of the starch.

As early as 1853 Boussingault distinguished between the hardening of bread due to the evaporation of water and that due to inherent or true staling as known today. He demonstrated that true staling is the more important and took place even when evaporation was prevented by storing the bread in hermetically sealed containers.

Horsford (1873) was apparently the first to suggest that the staling of bread is due to a change in the distribution of water between the starch and gluten. Lindet (1902) attributed staleness to what he called the "retrogradation" of starch. Katz (1913) proposed the hypothesis that there exists in the crumb of bread a physico-chemical equilibrium involving the state of the starch and believed that the rate at which staleness sets in is dependent on temperature. He found that at from 50° to 100° C. fresh bread is the equilibrium state and at from 25° to 0° C. stale bread is the stable form. He found no definite temperature at which bread became stale but noted the most rapid increase in the rate of onset of staleness between 50° and 25° C. Recently Alsberg (1936) has explained that as bread cools the soft starch jelly sets into a stiff but still elastic gel which contains less water than is necessary for complete gelatinization. The starch gel shrinks a little during the process of setting and for some time afterward. Because the gluten is losing moisture to either the starch or crumb solution, or both, it may also shrink and the strains which are set up at the gluten-starch interfaces ultimately cause them to separate and the bread becomes crumbly.

¹ Paper No. 1561, Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis submitted to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Degree of Master of Science.

² American Soya Products Fellow 1935-1937.

Platt (1930) confirmed Katz's observations upon the effect of temperature on bread staling and his experiments also indicated that lean formula bread became stale more rapidly than did rich formula bread. His bread made from the rich formula not only remained softer throughout the test but the richer bread had a slightly but significantly slower rate of staling than the lean bread. In an experiment conducted by Whympers (1919) it was found that, judged organoleptically, bread made from a dough to which extra gluten had been added had better keeping qualities than bread made from the regular dough.

Experimental

Two types of bread were employed in all of the staling studies. The regular bread was made with 100% flour, $1\frac{1}{2}\%$ salt, $2\frac{1}{2}\%$ shortening, 3% sugar, 5% yeast and (variable) water. The second type of bread included in addition to the ingredients listed above, $1\frac{1}{2}\%$ defatted soybean flour and $4\frac{1}{2}\%$ additional water. These two types of bread were given identical treatment during fermentation, baking and storage. The dough batches were of such size that all loaves of a particular type used in a single study were obtained from the same dough. The doughs were fermented for one hour at 30° C. with one kneading. After 35 minutes' pan proof at 30° C. the loaves were baked for 30 minutes at 230° C.

After cooling one hour at room temperature, measurements were made on one loaf of each type of bread and the remaining loaves were wrapped and sealed in wax paper and placed in storage. Fresh bread measurements were made on one loaf from each batch.

In this study three methods of measuring staleness were employed concurrently to follow the changes in the aging of bread crumb. A measure of the firmness of the crumb was made with an apparatus similar to that used by Platt (1930). This device measures the depression of the surface of a slice of bread crumb effected by a plunger acting under a unit force or weight. The second method was that of Katz (1928) and involved the determination of swelling power by measuring the volume of sediment obtained from 10 grams of finely ground bread crumb. The crumb of fresh bread occupies a greater volume than that of stale bread due to the greater water-absorbing capacity of the fresh bread starch. The third test employed Karacsonyi's (1929) method of measuring the viscosity of a 10% suspension of finely ground bread crumb. The use of this test as a measurement of staleness is also based on the swelling power of the starch.

Throughout this study the results which are shown are the average values of staling tests conducted on replicated loaves. After storage under the conditions which will be specified, the tests were repeated on

the aged bread, and the percentage reduction of these values from the fresh bread values were plotted against time of storage. The curves having the greatest slope and reaching the highest values thus represent the bread which has undergone the greatest amount of staling.

The Effect of Flour Strength on the Keeping Quality of Bread

Four flours of varying strength were selected to be used in this study of the effect of flour strength. These flours were commercially milled from the classes of wheat shown in Table I.

TABLE I

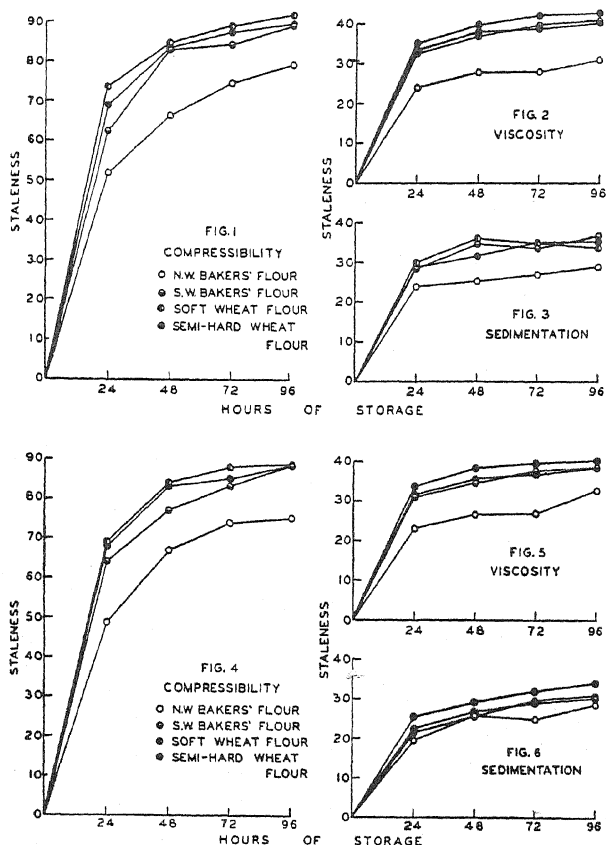
DESCRIPTION OF FLOURS USED IN STUDY OF EFFECT OF BAKING STRENGTH ON BREAD STALING

Flour number	Flour designation	Wheat from which flour was milled	Crude protein
			(N X 5.7) %
16699	Northwestern bakers' flour	Northwestern hard spring wheat	12.6
16700	Southwestern bakers' flour	Southwestern hard red winter wheat	11.6
16824	Soft wheat flour	Southwestern soft red winter wheat	10.1
16825	Semi-hard wheat flour	Nebraska semi-hard winter wheat	9.6

The data recorded in Figures 1 to 3 show the comparative rates of staling of the regular bread and those in Figures 4 to 6 show the staling of soy bread baked from these four flours and stored at 28° C. These staling curves show that the rate of staling of bread is dependent, in part, on the type of flour from which the bread was baked. In general, all three methods of measuring staleness indicated that the loaves baked from the stronger flours did not stale as rapidly nor did they reach as high a degree of staleness as those baked from the weaker, lower protein flours. However, keeping quality of bread does not appear to be strictly a linear function of protein content. Apparently protein characteristics or some other factor or factors inherent in the flour enter into the phenomenon of staling. There was a wider spread in the rates of staling of bread made from the northwestern bakers' flour and the southwestern bakers' flour than there was in the rates of staling of the southwestern bakers' flour and the soft wheat flour, although the difference in protein contents of the first two flours was only 1.0% and that of the second and third flours was 1.5%.

As measured by the compressibility test, the bread made from the 10.1% protein soft wheat flour staled more rapidly than did the bread

made from the 9.6% protein semi-hard wheat flour. This fact also points toward other factors in flour than protein content which account for the differences in keeping quality of bread made from various flours.

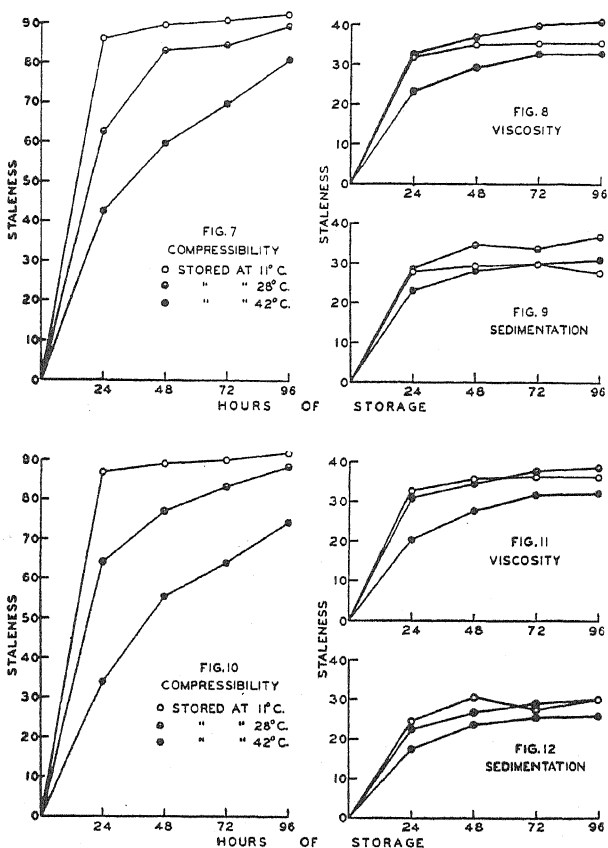


Figures 1 to 6. The effect of flour strength on the staling of bread.

The Influence of Temperature of Storage on the Rate of Staling of Bread

To study the effect of temperature on staling, bread baked from the southwestern bakers' flour was stored at 11°, 28° and 42° C. Storage at 11° C. is comparable to that often encountered in delivery trucks during the cooler seasons of the year. Temperatures of 28° C. are quite common in stores and homes, especially during the summer months. It was believed that storage at 42° C. would introduce the effect of relatively high temperatures on the rate of staling without causing excessive drying of the bread.

The graphs in Figures 7 to 12 show the extent to which temperature of storage affects the rate at which bread crumb becomes stale. The curves in Figures 7 to 9 represent the staling of regular bread baked from the southwestern bakers' flour and those in Figures 10 to 12 show the staling rates of soy bread. The compressibility test showed that



Figures 7 to 12. The effect of temperature of storage on the staling of bread.

the bread stored at 42° C. did not become as firm as that stored at 28° C. and the latter in turn did not lose compressibility as rapidly as that kept at 11° C. Both the rate of staling and the final degree of staleness as thus measured, even after 4 days' storage, became less as the temperature of storage was increased from 11° to 42° C. Bread stored at 11° C. had very nearly reached its maximum firmness after but 24 hours. On the other hand, bread stored as 42° C. was still quite fresh after 24 hours storage and even after 96 hours was not as firm as

that kept at 11° C. for 24 hours. The staling curves obtained from the data of the compressibility test indicate that as the temperature of storage is raised the staling of bread becomes more nearly a linear function of time.

Although the viscosimetric and sedimentation methods did not afford as consistent and uniform results as the compressibility test, the results obtained by the first two methods indicate that, in general, the lower storage temperature was more conducive to rapid crumb staling. The staling rate was not as rapid, especially during the early storage period, in the bread stored at the higher temperatures. However, at 72 hours' storage the staling rates, as determined by these methods which measure the swelling power of starch, converged closer to the same value. The curves of crumb compressibility did not appear to converge until after 96 hours' storage. In view of the fact that the compressibility method measures a somewhat different change in bread crumb from that measured by the viscosimetric and sedimentation methods, the above observation appears to indicate that the development of crumb hardness tends to lag behind loss of swelling power.

Effects of storage temperature similar to the above were also obtained with bread baked from the spring wheat flour.

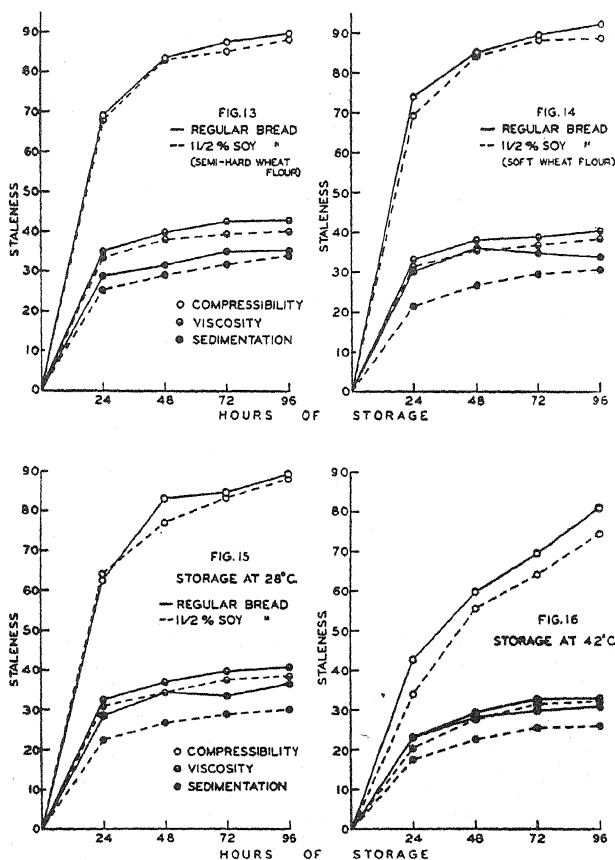
The Effect of Soy Flour on the Keeping Quality of Bread

To obtain a direct comparison of the rates of staling of the soy bread and the regular bread, the appropriate curves obtained in the foregoing studies are assembled in Figures 13 to 16. The addition of 1½% soy flour to the semi-hard wheat flour and to the soft wheat flour is shown in Figures 13 and 14 to effect some retardation of staling. This bread was stored at 28° C. The rate of staling of the soy bread is represented by the broken-line curves, and it will be observed these curves fall below those of the regular bread, indicating less staling in the soy bread.

Figures 15 and 16 show the effect of soy flour on the staling of bread baked from the southwestern bakers' flour and stored at 28° and 42° C. The difference in the rates of staling of the regular and soy bread was more pronounced when the bread was stored at 42° C. than at 28° C. At the higher temperature of storage the soy bread was fresher 4 to 24 hours after the regular bread had reached any particular stage of staleness.

The use of 1½% soy flour appeared to effect greater retardation in staling in the bread baked from the lower protein flours than that baked from the stronger flours. Although no tests were made it is quite probable that the use of a higher percentage of soy flour would effect a greater retardation of staling than was found with the use of 1½% of

this product. This retardation of staling obtained by the use of soy-beam flour is in agreement with the results reported by Straub and Hirsch (1935). Using a mechanical testing device, they found that



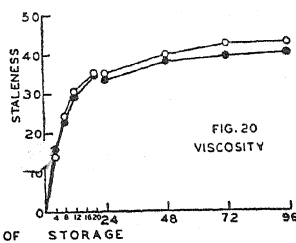
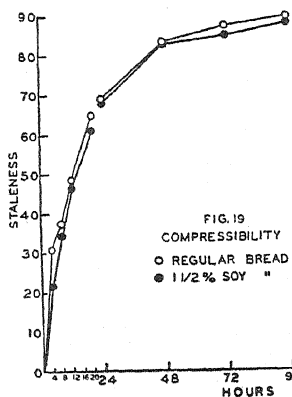
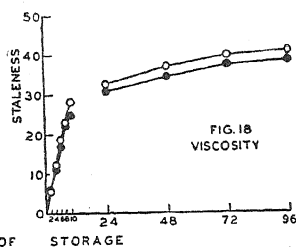
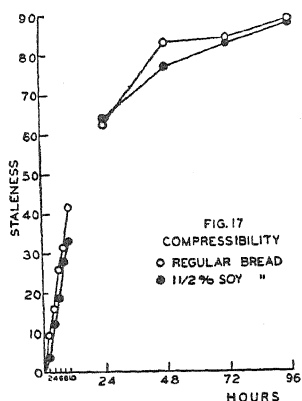
Figures 13 to 16. The effect of soy flour on the rate of staling of bread.

after one to three days' storage soy bread was softer than bread containing no soybean flour.

The Rate of Staling During Early Storage

Throughout these studies it has been observed that the most rapid staling takes place during the first 24 hours of storage. To study staling during early storage, tests were made on a series of loaves baked from the southwestern bakers' flour. Staling determinations were conducted on the bread one hour after baking and at two-hour intervals

of storage at 28° C. for a period of ten hours. The sedimentation method was not used in this study because it was not sufficiently accurate to detect the small differences which occur during these short intervals of time. In Figures 17 and 18 is shown the very rapid initial rate at which the bread staled. The curves of the rates of staling during the 24 to 96 hour period, as previously shown, were added to these figures to complete the picture.



Figures 17 to 20. The rate of staling during early storage.

The curves in Figures 19 and 20 show the rapid staling of bread baked from the semi-hard wheat flour. The most rapid staling was found to occur during the first 8 to 12 hours out of the oven, after which time the rate rapidly decreased.

A Comparison of the Methods Employed to Measure Staleness

All three methods gave results which agreed fairly well as to the relative rates of staling of the various types of bread studied. The staleness, after 24 hours' and 96 hours' storage, of the bread baked from the four types of flour as determined by one method was correlated with the staleness of the same bread as determined by each of the other tests. Similar correlations were made of the bread baked from the southwestern bakers' flour and stored at 11°, 28° and 42° C. The coefficients of correlation are shown in Table II.

TABLE II
CORRELATION OF METHODS OF MEASURING STALENESS

Methods being correlated	Correlation coefficient	
	At 24 hr. period	At 96 hr. period
Bread baked from four types of flour—storage 28° C.		
Compressibility and viscosity	+0.9270	+0.9045
Compressibility and sedimentation	+0.6146	+0.7251
Viscosity and sedimentation	+0.6974	+0.8437
Bread stored at three storage temperatures—southwestern flour		
Compressibility and viscosity	+0.8928	+0.6986
Compressibility and sedimentation	+0.6973	+0.3887
Viscosity and sedimentation	+0.8105	+0.7677

The coefficients of correlation of the compressibility and viscosity methods are fairly high; in three of the instances the correlations were found to be about $r = 0.9$, and in the fourth case the correlation was approximately $r = 0.7$. This indicates that these two methods disclosed approximately the same relative rates of staling of the various types of bread which were studied. The correlation of the sedimentation method to the compressibility test ranged from $r = 0.4$ to $r = 0.7$, whereas the correlation of the two methods of measuring crumb swelling was somewhat higher. In the latter case the correlation was $r = 0.7$ to $r = 0.8$.

The viscosimetric and sedimentation methods did not exhibit the wide spread in the rates of staling obtained by the compressibility method. The latter method showed greater effects of flour strength and temperature of storage than did the first two methods. Throughout this study the viscosimetric and compressibility methods gave fairly consistent results which produced reasonably smooth staling curves when calculated as percentage reduction from fresh bread values. Very few irregularities were obtained by these two methods. On the other hand, the sedimentation method often gave results which were quite different from those which were expected. This method is not

sensitive to small differences in the condition of the bread crumb and was of no value in attempting to follow the rate of bread staling during the early storage period. The experimental error of this method was greater than the differences found in the swelling capacity of the bread crumb between successive two-hour periods.

Summary

Three methods of measuring staleness were employed concurrently to follow the changes in the aging of bread crumb. A measure of the firmness of the crumb was made with an apparatus similar to that described by Platt (1930). This device measures the depression of a plunger into a slice of the bread crumb which is caused by the action of a standard weight on the plunger during a given period of time. Katz's method (1928) of determining swelling power by measuring the volume of sediment obtained from 10 grams of bread crumb was the second test employed. The method of Karacsonyi (1929) was also used to measure the absorbing capacity of the bread crumb. This method involves the measurement of the viscosity of a 10% suspension of finely ground bread crumb.

Flour strength appears to be an important factor in the aging of bread crumb, the rate of staling being a function, though not linear, of the protein content of the flour. Bread baked from the 11.6% protein southwestern winter wheat flour staled much more rapidly at 28° C. than did bread baked from the 12.6% protein northwestern spring wheat flour. Bread baked from the 9.6% protein semi-hard winter and the 10.1% protein soft red winter wheat flours staled more rapidly than did that of the southwestern flour, but this difference was not as great as the difference in the rates of staling of the bread baked from the two stronger flour types, even though the spread in protein content in the former case was greater than in the latter. That protein characteristics may be a factor in the staling of bread is indicated by the fact that, as measured by the compressibility method, the bread baked from the soft wheat flour staled more rapidly than did that baked from the semi-hard wheat flour.

Temperature of storage had a marked effect on the rate at which bread became stale. Bread stored at 11° C. staled very rapidly and was nearly as stale after only 24 hours' storage as it was after 96 hours' storage at the same temperature. Increasing the temperature of storage to 28° or 42° C. greatly retarded the rate of staling, and bread stored at the higher temperatures (28° and 42° C.) did not reach the degree of staleness at the end of the 96-hour period that was obtained at a storage temperature of 11° C. Increased storage temperature appeared to have a greater effect on the compressibility than on the

water-imbibing capacity of the crumb. At 42° C. the percentage decrease in compressibility was nearly a straight line function of time.

Soybean flour added to the bread dough tends to retard the staling of the baked bread. The use of 1½% fat-free soy flour was found to reduce both the rate and degree of staling of bread baked with various types of wheat flour. This retardation was more evident in the bread stored at the higher temperatures (28° and 42° C.); the soy bread remained fresh 4 to 24 hours longer at 42° C. than did the regular bread.

During the first 8 to 12 hours out of the oven, the bread was found to undergo the most rapid changes in crumb characteristics. After such intervals the decrease in compressibility and the decrease in water-absorbing capacity of the crumb were nearly linear functions of time. Following this initial period of rapid staling, the rate decreased rapidly.

Compressibility and viscosity measurements of staleness were more consistent and uniform than the data obtained by the sedimentation method. The effects of flour strength and temperature of storage were more pronounced in the compressibility measurements than in the data obtained by the other two methods. The sedimentation test is not sensitive to minor changes in the condition of the bread and is subject to error due to faulty settling of the crumb.

Literature Cited

- Alsberg, C. L.
1936 The stale-bread problem. Wheat Studies of the Food Research Institute. Stanford University, California.
- Boussingault, J. B.
1853 Ann. de chim. et de phys. III series 36, p. 490.
- Horsford, E. N.
1873 Report of the Commissioners of the United States to the International Exhibit Held at Vienna.
- Karacsonyi, L. P.
1929 A quick viscosimetric method for measuring the staleness of bread. Cereal Chem. 6: 241-243.
- Katz, J. R.
1913 The cause of the staling of bread. Z. fur Electrochemie 19: 202.
1928 Gelatinization and retrogradation of starch in relation to the problem of bread staling. A comprehensive survey of starch chemistry. Chem. Catalog Co., p. 100.
- Lindet, L.
1902 Sur les états que présente l'amidon dans le pain tendre et dans le pain rassis. Bul. Soc. Chim. de Paris, Serie III, XXVII, 634-639.
- Platt, Washington
1930 Staling of bread. Cereal Chem. 7: 1-34.
- Straub, J., and Hirsch, P.
1935 A method for judging the freshness of bread. Chem. Weekblad 32: 120-121.
- Whymper, R.
1919 The conditions which govern staleness in bread. Report to the British Gov't. Maclaren and Sons, Ltd., London.

A COMPARISON OF TWO SIZES OF BAKING PANS ON 100-GRAM FLOUR DOUGHS PRODUCING LARGE LOAF VOLUMES¹

H. MILLER and A. G. O. WHITESIDE

Cereal Division, Dominion Experimental Farm, Ottawa, Canada

(Received for publication January 17, 1938)

In evaluating the baking-strength qualities of small samples of wheat grown at the various experimental stations of the Dominion Experimental Farms System, the Cereal Division for some years has been using as the main baking formula the American Association of Cereal Chemists' standard baking formula with the additions referred to by Larmour, Geddes, and Whiteside (1933) of .001 g. KBrO_3 , .1 g. $\text{NH}_4\text{H}_2\text{PO}_4$ and .3 g. of a high diastatic malt extract (250° L.). This formula, when applied to high-protein flours milled from wheats grown in certain areas of the prairie provinces, has been giving loaves of exceptionally large volume; it is not uncommon for some of them to exceed 1,000 c.c. The low-form baking tin of the dimensions specified by Geddes (1934) has not been entirely satisfactory since the development portion of the loaves, classified by Blish (1928) as being the portion of the loaf above the pan, was much in excess of the base of the loaves, that portion within the pan, and the occurrence of large holes under the crusts at the top of the loaves was not unusual. Consequently, a number of baking pans of different sizes and shapes were employed to find out if more desirable test loaves might be produced by altering the size of the pan.

Experimental

After some preliminary trials in which a number of test bakes were made with the A. A. C. C. high and low pans and two other types slightly larger than the Pioneer pan described by Davis, Leatherock, and Putnam (1936), the pan hereafter described as the "large" pan was chosen for comparison with the A. A. C. C. low-form tin. The two pans have the following dimensions:

	A. A. C. C. low-form pan Cm.	Large pan Cm.
Top width	7.0	9.5
Bottom width	5.5	7.5
Top length	11.5	12.8
Bottom length	9.5	10.4
Height	5.0	6.5

¹ Contribution No. 107 of the Cereal Division, Dominion Experimental Farm, Ottawa, Canada

The approximate capacities are 350 c.c. and 650 c.c. for the A. A. C. C. low-form pan and the large pan respectively. The pans were both constructed from 2 xx tin.

Five flours, 2 commercially milled and 3 experimentally milled, were employed in the baking test using the above mentioned formula. The flour designations and protein contents are given in Table I.

TABLE I
PROTEIN CONTENT OF 5 FLOURS USED IN THE EXPERIMENT

Sample number	Flour protein (13.5% moisture basis)
	%
1	13.2
2	13.8
3	12.9
4	15.1
5	17.0

Samples No. 1 and No. 2 were commercial bread flours, the former a short patent and the latter a long patent flour. Samples 3, 4 and 5 were straight-grade flours milled in an Allis-Chalmers experimental flour mill. These five flours were selected for the range in loaf volumes which they afforded even though the baking formula and procedure were possibly not well suited for samples 1 and 2 since they were already chemically improved. Loaves were baked from these flours in both types of pans on each of 14 consecutive days, and in Table II the mean loaf volumes with their standard errors for the 14 bakes and coefficients of variability are given for each flour employing each of the two pans.

TABLE II
STATISTICAL DATA FOR LOAF VOLUMES OBTAINED WITH THE TWO PANS

Sample number	Mean loaf volume in c.c.		Coefficient of variability	
	A. A. C. C. pan	Large pan	A. A. C. C. pan	Large pan
1	587 $\pm$ 8.55	608 $\pm$ 12.03	5.5	7.4
2	735 $\pm$ 10.69	720 $\pm$ 9.35	5.4	4.9
3	859 $\pm$ 8.55	861 $\pm$ 8.55	3.8	3.7
4	938 $\pm$ 11.76	950 $\pm$ 5.35	4.7	2.1
5	1094 $\pm$ 9.35	1116 $\pm$ 8.82	3.2	3.0

It will be noted in Table II that no significant differences were obtained for the mean volumes of the loaves baked in the large pan over those baked in the A. A. C. C. low-form pan and that no improvement in replicability was shown for the former pan as indicated by

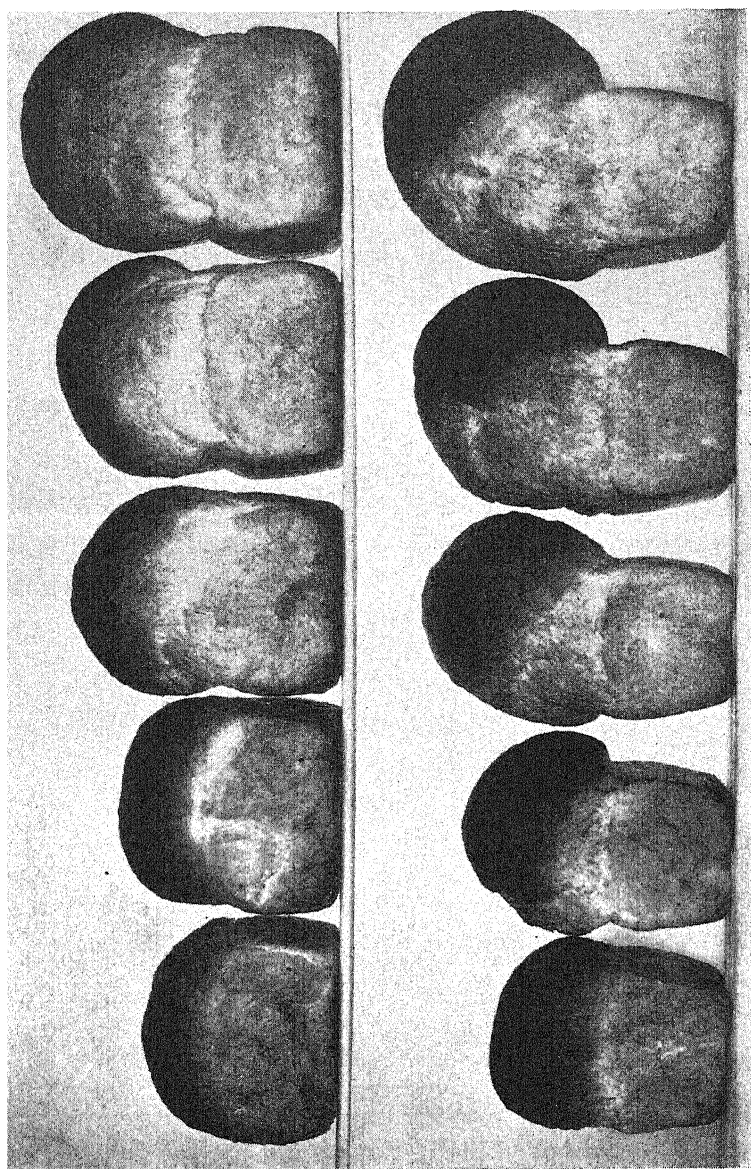


Figure 1. From left to right uncut loaves from flours Nos. 1 to 5. Top row baked in large pan; bottom row baked in A. C. C. low pan.

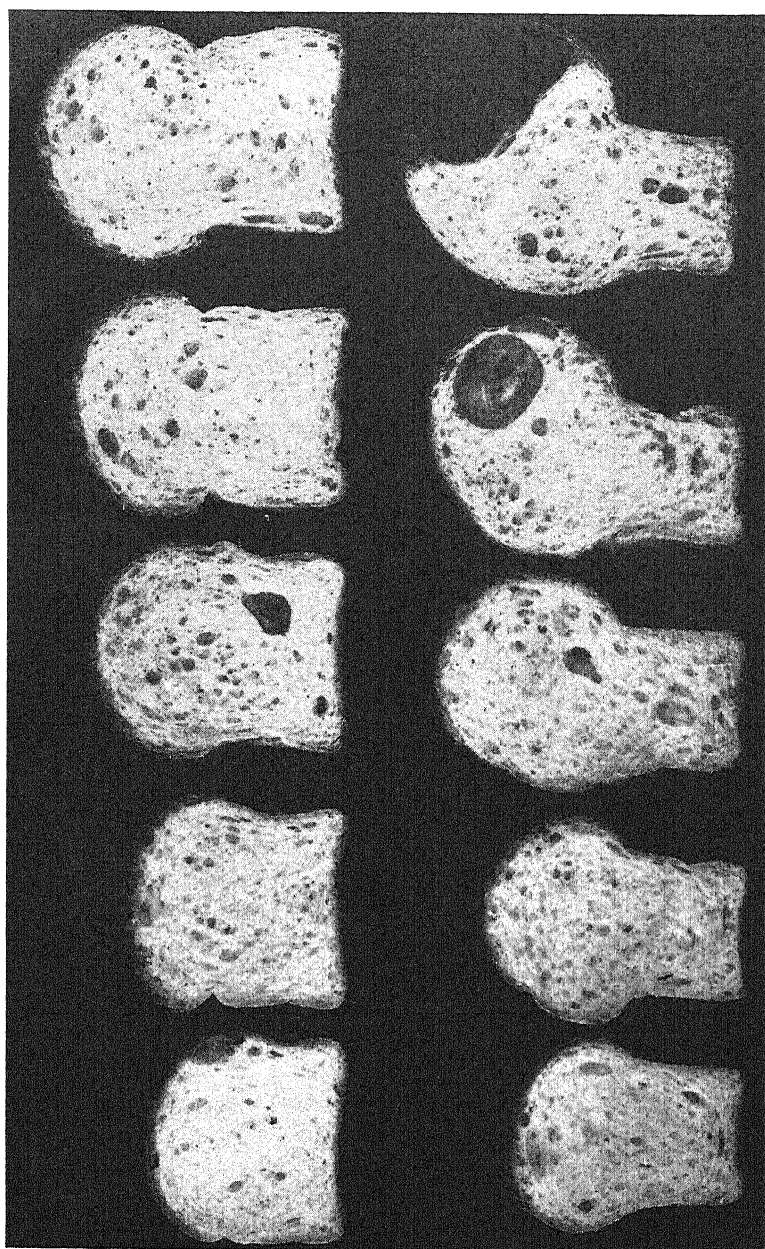


Figure 2. Cut loaves as in Figure 1.

the calculated standard errors or the coefficients of variability. This was surprising in view of the fact that the large pan did not produce large holes in the development part of the loaf although these occurred fairly frequently in the loaves baked from flours 3, 4 and 5 employing the A. A. C. C. low-form pan.

In Figures 1 and 2 the external and cut surfaces of the loaves for the 5 flours numbering from left to right, baked on one of the days, are represented. The superiority of the large pan for producing more uniformly shaped loaves, especially for flours 3, 4 and 5, is evident. Moreover, the tendency for the A. A. C. C. low-form pan to produce large holes in the development part of the loaf is shown for the loaves baked from flours 4 and 5. This tendency, it is believed, is due in part to the lack of protection given to the upper part of the proved dough by the smaller pan when doughs of very strong flours are first placed in the oven, as this fault has been corrected when the larger pan was used. The crumb structure of the loaves baked in the larger pan is inclined to be a little more open, but more uniform throughout, than when the smaller pan was used. The loaves such as those produced from flours 3, 4 and 5, baked in the smaller pan, produced crumb structures which are not uniform as the upper or development part of the loaf is more open than the lower or base part. It is, therefore, clear that the relation of the dough to the top of the pan at oven time influences both the internal as well as the external characteristics.

Summary

Two baking pans, the A. A. C. C. low-form tin and a larger pan, have been compared employing 5 flours of varying protein contents. For very strong flours the larger pan produced loaves of better external appearance, eliminated the tendency of large holes occurring under the crust, and gave more uniform crumb structures, although slightly more open, than did the smaller pan. The change in pan size did not alter, appreciably, the loaf volumes nor did the larger pan appear to improve the replicability of bakes on different days. As would be expected, the A. A. C. C. low-form pan gave more desirable appearing loaves with volumes under 700 c.c.

Literature Cited

- Blish, M. J.
1928 Proposed reporting system for standard baking test. *Cereal Chem.* 5: 289-294.
Davis, C. F., Leatherock, L. E., and Putnam, H. W.
1936 Experimental baking pans. *Cereal Chem.* 13: 113-118.
Geddes, W. F.
1934 Official A. A. C. C. baking test. *Cereal Chem.* 11: 363-367.
Larmour, R. K., Geddes, W. F., and Whiteside, A. G. O.
1933 Comparison of various baking formulas used in testing wheat quality the plant-breeder. *Cereal Chem.* 10: 601-604.

REPORT OF THE A. A. C. C. METHODS COMMITTEE

R. M. SANDSTEDT, *Chairman*

Department of Agricultural Chemistry, University of Nebraska, Lincoln, Nebraska

(Read at the Annual Meeting, May 1937)

General Report

The Methods Committee during the past year has developed and studied the following methods or adaptations of methods:

A method for sucrose in flour, a short gassing power method, a 15-minute moisture method for flour, and an adaptation of the ferricyanide method for high diastatic flours and for sucrose. The subcommittee on viscosity has continued its studies. An attempt has been made to compile and present to the Association various short-cuts and improvements in methods, and laboratory devices or "gadgets" which the members of the Association have developed and find useful but which are ordinarily not available to the general membership of the Association.

Recommendations as to Policy

It has been the policy of the Association to take no part in giving direction to the Methods Committee work. A new committee is chosen each year, each member of which selects his own problem for work. This is a "hand-to-mouth" policy which provides no definite objective. Accordingly it is recommended that the policy of the Association toward the Methods Committee be so changed that direction may be given to the committee work and the committee given definite objectives. This may be accomplished by selecting the methods to be investigated in advance of the appointment of the membership of the committee. The methods selected should be those for which the Association feels a need and the members of the committee should be chosen because of their special qualifications to work on these particular methods. This change in policy would place the members of the Methods Committee on a basis similar to that of the associate referees of the Association of Official Agricultural Chemists.

It is further recommended that the collaborative facilities of the Sections of the Association be used, with limitations, for further development of those methods recommended by the Methods Committee; that is, that in so far as the sentiment of the various Sections is favorable, the collaborative checking of the Sections be extended

to include those improved methods in which the particular Section is interested. This would greatly facilitate the improvement of methods by proving or disproving their value more speedily. It would also put desirable changes into general use more rapidly. It is also highly desirable that the other committees make use of certain recommended procedures in their work, *e.g.*, those using viscosity determinations should make use of the technique recommended by the subcommittee on viscosity.

Recommendations on Methods

It is recommended:

That the methods developed this year be further studied collaboratively.

That the collection and dissemination of knowledge concerning "short-cuts and gadgets" be continued.

That the following recommendations held over from last year's methods committee be given consideration.

"That more extended research be done relative to the evaluation of shortening, milk, malt, yeast, yeast foods and other ingredients which are used in breadmaking.

"That the subject of laboratory experimental milling be further studied and an effort made to approach more uniform conditions in laboratory milling procedures.

"That methods for the measurement of the proteolytic activity of flour be investigated."

ERRORS INVOLVED IN THE MEASUREMENT OF GAS PRODUCTION BY THE FERMENTOGRAPH

FREDERICK D. SCHMALZ and B. SULLIVAN

Russell-Miller Milling Company, Minneapolis, Minnesota

(Received for publication August 2, 1937)

Introduction

The Fermentograph is one of the instruments in use for the determination of gas production in doughs. This instrument employs the law of buoyancy in recording the volume of gas evolved in a dough; a body immersed in a liquid is buoyed up by a force equal to the weight of the displaced liquid in accordance with Archimedes' principle.

The Fermentograph has the advantage of automatic graphic recording of the amount of gas produced. However, various factors interfere with the true recording. The machine is of great use for routine tests but for high precision work leaves much to be desired.

The Brabender Company¹ lists the following factors to be considered in the calibration of the instrument:

1. The weight of carbon dioxide in the balloon.
2. The change of the specific weight of water at 30° C. and 4° C.
3. The compression of the carbon dioxide in the rubber balloon and in the dough medium.
4. The pressure of the water column on the rubber balloon and the consequent change in volume of gas.

In addition to the factors enumerated above, our experience with the Fermentograph has shown that other important causes of error must be taken into account. These are:

1. Diffusion. Carbon dioxide is a water soluble gas, and rubber is a permeable membrane allowing the gas to diffuse and dissolve in the water bath.
2. Stretching of the rubber balloon.
3. Punching of the balloon.
4. Mechanical defect in the balance head.

First of all, it was found that when identical doughs were placed in all four units, the checks were not as close as was desired. The scale

¹ Private communication.

heads were then checked one against the other and mechanically adjusted to give uniform readings from unit to unit. Another experiment was conducted in which doughs were prepared with identical formulas, but the quantity of dough placed in each balloon was varied. One would expect to obtain double the amount of gas if one had double the amount of dough, but this was not the case. From experiments such as these, it was concluded that an attempt should be made to evaluate the individual errors and measure, as far as could be done, the quantitative effect of each error.

Discussion

Weight of carbon dioxide in the rubber balloon. The maximum amount of carbon dioxide present at any one time can not be more than 1000 c.c. If 22.4 l. of carbon dioxide weigh 44 g., one liter would weigh $\frac{44}{22.4}$. This would be less than 2 g. and since it would record about 2 c.c. it is a negligible error. Carbon dioxide, of course, is not the only gas present since alcohol, water vapor and some volatile organic compounds comprise a portion of the gas. These latter, however, are not present in any significant quantity.

The specific weight of water. The density of water at 30° C. is 0.995646 or 1000 c.c. are buoyed by a force equal to 995.6 g. and not 1000 g.

Compression of carbon dioxide in the rubber balloon and in the dough. No attempt was made to evaluate this factor since it was not known just what was meant.

Pressure of the water column on the balloon and the consequent change in volume due to this additional pressure. The balloon is immersed in the water so that its top is about 2 inches from the surface of the bath and its bottom about 8 inches from the surface. The rise of the balloon in traversing the entire range of the 1000 c.c. on the graph is approximately three-quarters of an inch. Therefore, after the development of 1000 c.c. of gas the balloon is closer to the surface by about three-quarters of an inch than it was at zero cubic centimeters. For the sake of a rough computation, let five inches be taken as the average length of the column of water over the balloon. The balloon is then under a pressure of the atmosphere plus 5 inches of water. Five inches of water are equivalent to approximately 9.5 mm. of mercury and thus the pressure on the balloon is 760 plus 9.5. Therefore, 1000 c.c. immersed under a column of 5 inches of water become 987. The variation in pressure due to the rise of the balloon can not affect the volume by even one cubic centimeter.

EXPERIMENTAL

Diffusion

The error due to diffusion is large and the measurement of it was attempted in the following manner. Carbon dioxide gas developed during the fermentation in a dough-filled balloon was used to inflate an empty balloon. The balloon filled with carbon dioxide gas alone was placed in its holder and suspended in the water bath. The loss of gas was noted by the fall of the pointer on the graph. The loss varied with the amount of gas in the balloon; about 30 c.c. diffused through the balloon in an hour when it was inflated to approximately 150 c.c. of gas and 60 c.c. diffused when the gas volume in the balloon was 1000 c.c. In order to be sure that the loss was due to the solubility of carbon dioxide and could not be attributed to leaks in the balloon, air was substituted for the carbon dioxide. No loss of air was observed.

It was hoped that a heavier rubber might stop the diffusion or reduce it to a negligible minimum. With this in view, a home-made balloon was constructed from automobile inner tubes and tire patch. The diffusion was not stopped but was considerably lessened; it amounted to about 16 c.c. per hour, constant more or less over the entire range. Experiments on the inner tube balloon showed that diffusion, even when it is diminished by use of the heavier rubber, remains quite an error. Identical doughs, except for size, were fermented in the heavy rubber balloon. A reading of 551 c.c. was recorded for a 100-g. dough, and 868 c.c. for a 150-g. dough. Three halves of 551 is 825 and this figure falls far below the figure actually obtained for the 150-g. dough. However, if a correction is made for diffusion, more reasonable results are obtained. The doughs were fermented for a four-hour interval and according to a diffusion factor already determined, the doughs lost 64 c.c. by diffusion during this time interval. When this amount is added to each figure, 615 c.c. are obtained for the 100-g. dough and 932 c.c. for the 150-g. dough. If 615 is multiplied by $\frac{3}{2}$ a figure of 923 is found. This is only 9 c.c. from the figure of 932 for the 150-g. dough, a fair experimental error.

A further proof that the diffusion is due to the solubility of carbon dioxide in the water was shown by experiments in which the dough was fermented in lubricating oil baths and saturated salt solutions only replacing them in the regular water bath at the hourly intervals to register the volumes on the graph. Between 4 and 5% more gas was obtained by fermenting in such media than in water.

There are two methods by which diffusion could be eliminated, one to make the balloon out of a material that is impermeable to the carbon dioxide and the other to substitute some liquid, in which carbon dioxide

is insoluble, for the water in the constant temperature bath. The difficulty of substituting some material for the rubber is in the manufacture of the article. Duprene, for example, is known to be less porous to gasses than ordinary rubber, but one can not obtain Duprene balloons of the size and shape needed. The ideal liquid for the bath should have these properties: It should not dissolve carbon dioxide, it should have a density of one, should not harm rubber, nor be prohibitive in price.

Stretching of the Rubber

An error of some magnitude can be introduced by the stretching of the rubber balloon. The capacity of the balloon unstretched is about 1100 c.c. If a dough weighing 400 g. is placed in the balloon it can be seen that in order that 1,000 c.c. be recorded, the balloon must be stretched. Experiments showed that an expanded or stretched balloon recorded 20 c.c. less than an unstretched balloon for a 400 c.c. volume.

Punching of the Balloons

It is recommended by the manufacturers to punch down the dough at the end of each hour and expel the gas from the balloon. This is necessary if a sizable dough is used in order to make room for the gas developed during the later hours of fermentation. This can be circumvented by using a smaller dough so that the total gas developed over the entire fermentation does not exceed 1000 c.c. Then the balloon need not be punched but in this method other errors such as diffusion are great relative to the dough size.

In punching the balloon, between $1\frac{1}{2}$ and 2 minutes are required. This is a 2-minute loss between every hour of dough fermentation and in 6 hours amounts to 10 minutes. Though the loss in time can be kept quite constant, the loss in unrecorded gas volume is an uncontrollable figure. At the height of fermentation a dough may be producing 16 c.c. per minute and in the later stages only 3 or 4 c.c. With this variation in the rate of gassing power, the gas loss can not be kept constant or easily computed when different flours and different formulas are used.

Mechanical Defect in the Balance Head

In order to determine how the machine recorded with known amounts of gas, the following set-up was used. A gas burette measured successive increments of gas placed in the balloons and the Fermento-graph recording was noted. The gas used was air. The temperature regulator on the bath was turned off to eliminate a temperature error of measuring the gas at room temperature in the gas burette and at 30° C. in the water bath.

For an interval from 0 to 500 c.c. the Fermentograph averaged 79 c.c. for each 100 c.c. of air, and from 500 to 1100 c.c. it averaged 90 c.c. It can be seen that the recording is not uniform from one end of the graph to the other. The lower readings on the Fermentograph can be accounted for by the fact that Brabender states the machine has been calibrated to reduce the readings to standard conditions of temperature and pressure. Doing this we obtain:

Temperature.....25° C.
Barometer.....737 mm.

$$\frac{1000}{1} \times \frac{737}{760} \times \frac{273}{273 + 25} = 888.$$

For 1000 c.c. of air, the machine records 870 c.c., an error of 18 c.c. Unless each machine is adjusted individually to compensate for altitude differences under which each will be operating, the mechanical computation for reduction to standard conditions will never be correct.

Summary

An evaluation of the errors involved in the measurement of gassing power by the Fermentograph is given. From the results of our work all the errors, except diffusion, can be overcome successfully and compensated for, if the machine is calibrated with known amounts of gas. The diffusion factor, however, is too large an error to be disregarded, and in order to make the Fermentograph entirely reliable the diffusion factor should be taken into consideration.

THE MARCH OF EXPANSION AND TEMPERATURE IN BAKING BREAD¹

C. H. BAILEY and EMIL MUNZ

Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota

(Received for publication March 8, 1938)

The march of temperature in bread dough during ordinary oven baking has been the subject of several studies reported in the literature. Rate of temperature increase has been demonstrated to be a function of the size of the dough mass, as well as of the temperature of the oven. It has appeared, generally, that the temperature finally attained by the interior of the loaf is in the range of the temperature of boiling water at the barometric pressure where the baking is done. In none of these earlier studies, as far as we are aware, has the march

¹ Paper No. 1596, Journal Series, Minnesota Agricultural Experiment Station.

of temperature been related with the relative expansion of the dough. The studies here reported were accordingly directed toward that end.

Our first investigations were undertaken with pan bread, that is, with dough baked in an iron pan. The relative dimensions of the pan are indicated by the cross section of the baked loaves shown at the top of Figure 1. Near the bottom of the pan the slices were 10 cm. across, increasing at the maximum to 12 cm. as shown in the diagram. Vertically the slice approached 15 cm. in height. The quantity of dough was 500 g. in each instance.

At the time of molding or forming the doughs before placing them in the pans, thermocouples were inserted into the interior of the mass. The position of these thermocouples at the conclusion of baking was noted and is recorded by small open circles in Figures 1 and 2. These thermocouples were copper-constantan, with two hot junctions in series in the dough and the cold junction in melting ice ($0^{\circ}\text{C}.$) in a thermos bottle. The potential of this multiple thermocouple was measured by means of a pyrovolter, and the values in E.M.F. converted into terms of $^{\circ}\text{C}.$ after careful calibration of the thermocouples. The doughs were "proofed" or allowed to ferment in the pan at $30^{\circ}\text{C}.$ in the customary manner, and then placed in an electrically heated oven maintained as closely as possible at a temperature of $200^{\circ}\text{C}.$

Under these conditions, in an oven in which the water-vapor is trapped so that the oven atmosphere is relatively humid, a dough made with strong flour, properly fermented, will expand or "spring" considerably. Thus a typical dough made from strong, high-protein flour and weighing 500 g. had a displacement of 1670 c.c. when placed in the oven, and of 3040 c.c. when baked, or an increase in volume of 1370 c.c., equivalent to 82%.

It was impossible to measure conveniently the actual displacement of the dough at intervals during the baking process. In lieu of this, a device was arranged in the oven by means of which the relative height of the dough could be observed at any time. The changing height is approximately proportional to the increase in volume, at least to the extent of enabling us to estimate the relative increase in displacement during baking. Such observations were made in the instance of three series of pan loaves, concurrently with the measurements of temperature by means of the thermocouple, and are recorded in Figure 1.

In the instance of loaves No. 1 and No. 2 where the thermocouples were about half way between the surface and the center of the loaf, the initial rate of temperature increase was somewhat greater than in the center of loaf No. 3. The vertical expansion of these same loaves,

recorded by the curves marked "E," was most rapid during the first 5 or 6 minutes of baking when the interior of these loaves was in the range of $40 \pm ^\circ \text{C}$. Expansion was practically concluded at the end of the 10th minute when the interior of the same loaves was in the range of 50° to 60°C . Thus the actual increase in dough temperature was only 20° to 30° when expansion ceased, although the dough still increased in temperature 40° to 50° more before the baking process was concluded.

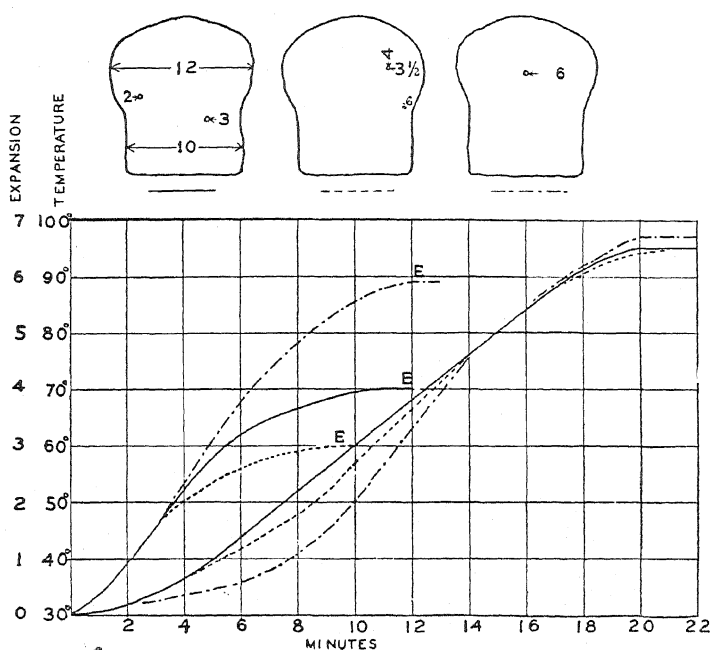


Figure 1. Observations on pan bread during baking. Curves E record expansion in centimeters measured in vertical height. Curves terminating at the right record the changes in temperature in the interior of the doughs during baking at 200°C , temperatures being measured at the positions shown in the sections illustrated alone and with the distance in centimeters from the outer crust being shown in each case.

Another series of experiments was then conducted with "hearth bread," that is, loaves baked on the hearth of the oven and not enclosed in a pan. Measurements in this instance are not as simple or satisfactory as with pan bread where the expansion is directed vertically to a considerable degree. The observations, as recorded in Figure 2, are of the same general nature as those made with pan bread, although expansion continued for about 2 to 4 minutes longer and the rate of temperature increase was somewhat slower. The cessation of expansion came at about the same temperature, namely $60 \pm ^\circ \text{C}$.

These observations lead to some speculation respecting the physical and biochemical bases for dough expansion in baking. The following factors might be proposed:

1. Thermal expansion of gases held as such in the vesicles of the dough.
2. Distillation of CO_2 from the dough solution and its expansion with the elevation of temperature.
3. Distillation and expansion of water vapor.
4. Accelerated diastatic activity and fermentation rate in the dough as the temperature is elevated into the range of 55°C. , above

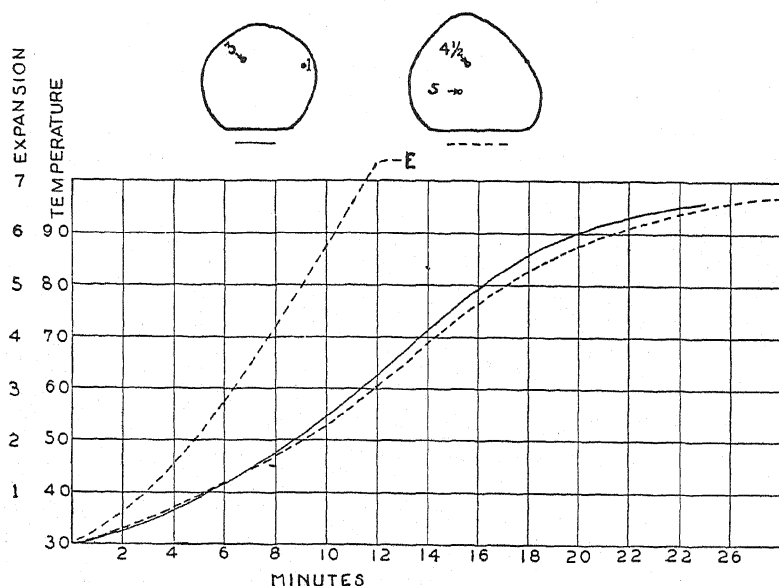


Figure 2. Observations on hearth bread during baking (see legend under Figure 1 for details).

which temperature the rate of such enzymic processes may be progressively reduced.

5. Changes in dough mobility or consistency which may facilitate expansion as induced by the factors outlined in 1 to 4.

It is not easy to measure accurately the individual contributions made in each of these instances. Approximate calculations involved in 1 and 2 would suggest that they were scarcely adequate to account for all the expansion of dough in baking. It must be conceded, however, that we do not know how much CO_2 is dissolved in the dough solution. Accelerated enzymic activity cannot be overlooked as a contributing factor when dough is heated progressively in baking.

Using the farinograph as a means for measuring the influence of temperature upon dough consistency, a series of observations was made at temperatures from 30° C. to 80° C. All these doughs were mixed with the same ratio of flour to water and consequently had the same mobility at 30° C. Two series of doughs were prepared with (1) a low-diastatic flour and (2) the same flour to which 0.37% of

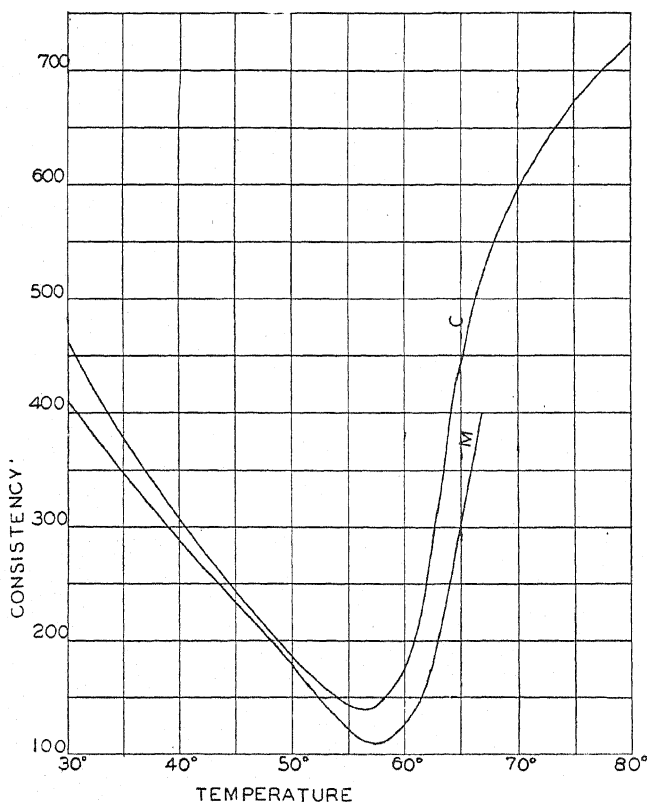


Figure 3. Relative consistency of doughs, measured with the farinograph, as a function of temperature. Curve marked C represents a simple flour-water dough, while curve marked M represents the same flour to which 0.37% of wheat malt had been added.

wheat malt had been added. The changing consistencies of the doughs in farinograph units, as a function of temperature, are recorded in Table I and graphed in Figure 3. It will be observed that the dough containing malt was more mobile than the reference or control dough. In general, the doughs became more mobile, *i.e.*, registered a lower farinograph unitage, as the temperature was increased to 57° C. With further elevation of temperature, the mobility decreased.

It is conceivable that the increased mobility of dough may facilitate expansion in baking. Certainly it is notable that expansion continued through that temperature range in which the dough was more mobile than when first placed in the oven.

As heating continues during the operations of baking, the dough becomes decidedly less mobile, notably between 62° and 67° C., and ultimately assumes a more or less rigid state in consequence of the heat denaturation or coagulation of the proteins, and the gelatinization of the starch.

TABLE I
RELATIVE MOBILITY IN FARINOGRAPH UNITS AT DIFFERENT TEMPERATURES

Temperature °C.	Malt-free flour dough	Flour +.37% wheat malt in dough
30	460	410
34	410	360
35	360	330
38	340	—
40	310	300
42	290	—
45½	250	230
47	215	—
48	210	200
51½	170	160
57	140	110
62	220	160
67	570	400
80	720	—

Summary

Oven expansion or "spring" involving the increase in volume or cubical displacement of pan bread amounted to about 80% of the dough displacement when first placed in the oven. This expansion was rapid during the first 10 minutes of baking, and ceased at about the time the median temperature of the dough passed through 50° to 60° C.

Median dough temperatures continued to rise during the next 10 or 12 minutes, however, and ultimately reached the temperature of boiling water after the 20th or 22nd minute.

Hearth bread behaved similarly except that the oven expansion or spring continued about 2 to 4 minutes longer, and the rate of temperature increase was a little slower.

Oven expansion is attributed to a combination of thermal expansion of gases or vapors present in or distilled from the dough, acceleration of enzymic phenomena in the early stages of baking which results in increased gas production, and all of these possibilities facilitated by increased dough mobility up to the time the dough becomes rigid in consequence of protein denaturation and gelatinization of the starch.

BOOK REVIEWS

Basic German for Science Students. Third Edition. By M. L. Barker. Published by the Chemical Publishing Co. of N. Y., Inc., New York, N. Y. 186 pages. 1937. Price \$2.50.

Dr. Barker's fundamental idea is to have the student learn, through the medium of his book, a reading knowledge of German adequate to give in English the gist of the German passage set in his Final B.Sc. paper (Great Britain) and to understand German articles and periodicals dealing with his special subject or research work. The demand for a third edition is evidence that its objective is being met. In this new edition is incorporated more of the recent passages for translation in the B.Sc. examinations of the Universities of London and Edinburgh.

The Introduction and Part I provide the student with the material necessary for acquiring a vocabulary of approximately 650 words of the first thousand basic words, *i.e.*, those words which are actually of most frequent occurrence in the writings of the German people.

The discussion of the German grammar has been reduced to the absolute minimum necessary for acquiring a reading knowledge of German.

The chief aim of Part II is to provide the student with a wide range of short scientific extracts, including chemistry, zoology, botany, physics, mathematics and medicine. In addition, the study of the first thousand basic German words and their most common derivatives is continued. Taking Parts I and II together, the total number of basic German words (including common derivatives) contained in the passages is approximately 1400.

Dr. Barker has presented in his book a satisfactory means for introducing the student into a knowledge of scientific German, but emphasis should be placed on the statement found in the introduction to the first edition, namely, that the book "will gain vitality and effectiveness in the hands of a teacher."

F. L. DUNLAP

The Determination of the Amino Acids. By Richard J. Block. Published by Burgess Publishing Co., Minneapolis, Minnesota. 85 pages. Price \$2.00.

The author gives all essential details of the selected methods for the determination of arginine, histidine, lysine, tyrosine, tryptophane, dihydroxy phenylalanine, thyroxine, proline, hydroxy proline, cystine, cysteine, methionine, glutamic acid, hydroxy-glutamic acid, aspartic acid, alanine, glycine, leucine, phenylalanine, and serine. In several instances the amino acids are considered in groups having related properties. Several methods are given for certain amino acid determinations when useful alternative procedures are available. The author has appended many useful footnotes to each chapter, including detailed descriptions of methods for the preparation of reagents, precautions to be observed at various stages in the determinations and other pertinent suggestions to smooth the path of the less experienced worker who is venturing into this field for the first time. In fact, Dr. Block gives such straightforward procedures as to deprive protein studies of much of their terrors, and the only caution the reviewer would sound is that possibly certain of these procedures are not quite as easy, nor as precise as might appear from reading the manual.

The final chapter of the book (Chapter XI) is comprised of outlines or "flow sheets" for the separation of amino acid mixtures including I. Fischer method, II. Cherbuliez method, III. Dakin method, IV. Brazier method, and V. Method of Przylecki and Kasprzyk. The reviewer suggests that this section of the book permits of considerable useful expansion to cover more details of these fractionation techniques, and precautions which must be observed at various stages.

Dr. Block has rendered a real service to workers in this field in organizing such a convenient manual. It is to be hoped that he will continue with this task, and will later add to the manuscript as new methods become available.

C. H. BAILEY

ANNUAL REPORT OF TREASURER

OSCAR SKOVHOLT

January 1, 1938

The Treasurer's Annual Report is presented in the same simple though complete form as established by previous incumbents. A continuing gain in membership is shown, although the increase is smaller than in the previous year. The surplus from the year's operations is greater. Only a few points will be made by way of comment:

In the statement of membership, the listing of "Members lost by death" has been substituted for "Members deceased during the year." The reason is that the death of a member that occurs during a year will not be reflected as a loss in membership until the following year if the current dues have been paid.

The 1937 Convention in Minneapolis was so conducted that a substantial surplus was unintentionally accumulated which was donated to the Association. The Executive Committee voted to place enough of this money into the Convention Reserve Fund to bring this to an even \$1,000.00 in amount. In the opinion of the Committee, this may be a sufficient reserve in this fund. The remainder of the surplus was expended in increasing the size of the 1937 volume of Cereal Chemistry.

The reprinting of Volume I, No. 1 of Cereal Chemistry was an extraordinary item of expense that was paid out of the year's receipts with a small surplus remaining to increase the reserve of the Cereal Chemistry fund.

The increase in surplus of general association funds was larger than usual due to the Convention surplus and the lack of any extraordinary expense except that of the Kansas City Exhibit.

"Cereal Laboratory Methods" nearly maintained the rate of sale of the previous year. At the close of the year, a total of 81 bound volumes was on hand with a further supply of 137 volumes at the printer's in unbound form. The rate of sale in recent months indicates that the supply may be exhausted by about the end of 1940, although unforeseen developments may change this date in either direction.

In the statement of the Distribution of Net Assets, entries have been made of *Interest Earned During 1936* that were not shown on last year's statement.

Savings accounts are distributed more widely than advisable for convenience even when due consideration is given to safety. The assets in Building and Loan Stock in Kansas City are carried at the reduced rate as authorized in 1936. Some reorganization is being completed that will result in a transfer of these assets to another concern, but their actual value cannot yet be determined.

DETAILED MEMBERSHIP STATEMENT DECEMBER 31, 1937

	Total	Active	Corp.	Hon.
Membership December 31, 1936.....	539	490	47	2
New members added during 1937.....	43	41	2	—
Members reinstated during 1937.....	12	12	—	—
Members resigned and suspended for non-payment of dues during 1937.....	29	27	2	—
Members lost by death.....	1	1	—	—
Members in good standing as of December 31, 1937	564	515	47	2
Net increase in membership during 1937.....	25	25	—	—

PROFIT AND LOSS STATEMENT

January 1 to December 31, 1937

RECEIPTS

Cereal Chemistry

Membership Dues

Active.....

Corporation.....

\$1,809.50

470.00

Subscriptions, reprints, back issues and advertising.....	\$6,775.88	
1937 Accounts Receivable.....		
Less: 1936 Income received in 1937.....	1,166.96	
Net 1937.....		5,608.92
Interest on Invested Funds.....		56.81
Surplus from Minneapolis Convention.....		287.34
Total Net Receipts 1937.....		\$8,232.57
Association.....		
Membership Dues.....	1,802.50	
Application Fees.....	123.00	
Interest on Invested Funds.....	64.66	
Surplus from Minneapolis Convention.....	350.84	
Miscellaneous Income.....	7.20	
Total Net Receipts 1937.....		2,348.20
"Cereal Laboratory Methods" Sales during 1937.....	387.90	
Interest on Invested Funds.....	9.30	
Total Net Receipts 1937.....		397.20
Decennial Index.....		
Received from Cereal Chemistry and Association.....		162.50
TOTAL RECEIPTS OF ALL ACCOUNTS 1937.....		\$11,140.47

DISBURSEMENTS

Cereal Chemistry.....		
Cost of printing Journal and Reprints.....	\$7,255.97	
1937 Accounts Payable.....	63.83	
Less: 1936 Accounts paid in 1937.....	1,158.70	
Net Cost of Printing.....		\$6,161.10
Cost of Editing and Miscellaneous Expenses....		1,690.89
Reprinting of Volume I, No. 1 Cereal Chemistry		155.00
Decennial Index—Cereal Chemistry's 1937		
Assessment.....		81.25
Net Disbursements 1937.....		8,088.24
Surplus 1937.....		\$ 144.33
Association.....		
Expenses of President's, Vice-President's Offices		
and News Letter.....	349.58	
Expenses of Secretary's Office.....	258.59	
Expenses of Treasurer's Office.....	198.36	
Committee Expenses.....	69.58	
Minneapolis Convention Report.....	316.90	
Decennial Index—Association's 1937 Assessment		81.25
Expenses of Kansas City Exhibit.....	55.91	
Miscellaneous Expense.....	27.00	
Net Disbursements 1937.....	1,357.17	
Surplus 1937.....		991.03
"Cereal Laboratory Methods".....		
Printing and Mailing Expenses.....	104.65	
Surplus 1937.....		292.55
Decennial Index.....		
Surplus 1937.....		162.50
TOTAL DISBURSEMENTS OF ALL ACCOUNTS.....	\$9,550.56	

DISTRIBUTION OF NET ASSETS

Cereal Chemistry Assets 1936.....	\$3,829.74	
Surplus 1937.....	144.33	
Interest Earned 1936—Not previously entered.....	7.50	
Assets December 31, 1937.....		\$3,981.57
Association Assets 1936.....	3,156.82	
Surplus 1937.....	640.19	
Interest Earned 1936—Not previously entered.....	21.53	
Assets December 31, 1937.....		3,818.54
Convention Reserve Fund 1936.....	649.16	
Surplus 1937.....	350.84	
Assets December 31, 1937.....		1,000.00
Cereal Laboratory Methods Fund 1936.....	777.74	
Surplus 1937.....	292.55	
Assets December 31, 1937.....		1,070.29
Decennial Index Fund 1937 Deficit.....	162.50	
Surplus 1937.....	162.50	
Assets December 31, 1937.....		None
Experimental Laboratory Baking 1936.....	80.95	
Assets December 31, 1937.....		80.95
TOTAL ASSETS DECEMBER 31, 1937.....		\$9,951.35

FINANCIAL STATEMENT DECEMBER 31, 1937

ASSETS

Manufacturers Trust Co.—Checking Account.....	\$3,481.91
Petty Cash Fund—Washington, D. C.....	200.00
Emigrant Industrial Savings Bank—New York.....	834.44
Franklin Savings Bank—New York.....	511.78
Harris Trust Company—Chicago.....	1,987.05
Building and Loan Stock—Kansas City ¹	1,000.00
U. S. Treasury Bonds.....	2,000.00
GROSS ASSETS.....	\$10,015.18

LIABILITIES

1937 Accounts Payable.....	63.83
NET ASSETS.....	\$9,951.35

REPORTS OF THE AUDITING COMMITTEES

We have examined the books and the report of the Treasurer for the year 1937 and to the best of our knowledge and belief, these are a true and accurate account of the receipts and expenditures of the American Association of Cereal Chemists.

H. K. PARKER, *Chairman*
CHAS. A. GLABAU
W. E. STOKES

We have examined the books of the Managing Editor of CEREAL CHEMISTRY for the calendar year 1937 and find the same to be correct to the best of our knowledge.

J. A. LECLERC
L. H. BAILEY

¹ Carried on the books at 50% of face value by order of Executive Committee of 1936 pending liquidation of Bankrupt Association.

CEREAL CHEMISTRY

VOL. XV

JULY, 1938

No. 4

SOME RESULTS WITH A NEW RECORDING MIXER FOR USE WITH SMALL SAMPLES ¹

J. G. MALLOCH

Division of Biology and Agriculture,
National Research Laboratories, Ottawa, Canada

(Read at the Annual Meeting, May 1937)

Introduction

There is urgent need for a reliable test for baking quality that can be used by plant breeders in the early stages of the development of their new strains of wheat. The quantity of grain available precludes the use of milling and baking tests or of dough-testing machines such as the Brabender, Swanson, Chopin or Buhler. The dough-ball test uses only small quantities of material but it is far from precise.

Considering the three general factors controlling baking quality, protein content and gassing power can be readily determined on small samples but gluten quality can only be inferred from gluten washing, baking tests or mechanical tests. The determination of physical properties seemed to offer the greatest promise of adaptability to small samples. Accordingly a recording mixer, operating with samples containing 7 g. of dry matter, was designed and constructed. Preliminary tests of its accuracy and utility are reported.

Description of Mixer

A general view of the machine is shown in Figure 1. There is a stainless steel spindle mounted on a vertical shaft, driven at 120 R.P.M. by a 1/20 H.P. synchronous motor. This spindle rotates inside a water-jacketed stainless steel cup which is free to rotate against spring tension. Figures 2 and 3 show the cup and spindle in greater detail. The spindle has three curved outer pins and a straight centre pin while the cup has two curved pins which are located between the centre pin and the outer pins of the spindle when the mixer is assembled. The purpose of the curvature of the pins is two-fold:

¹ Published as Paper No. 132 of the Associate Committee on Cereal Research of the National Research Council of Canada and the Dominion Department of Agriculture.

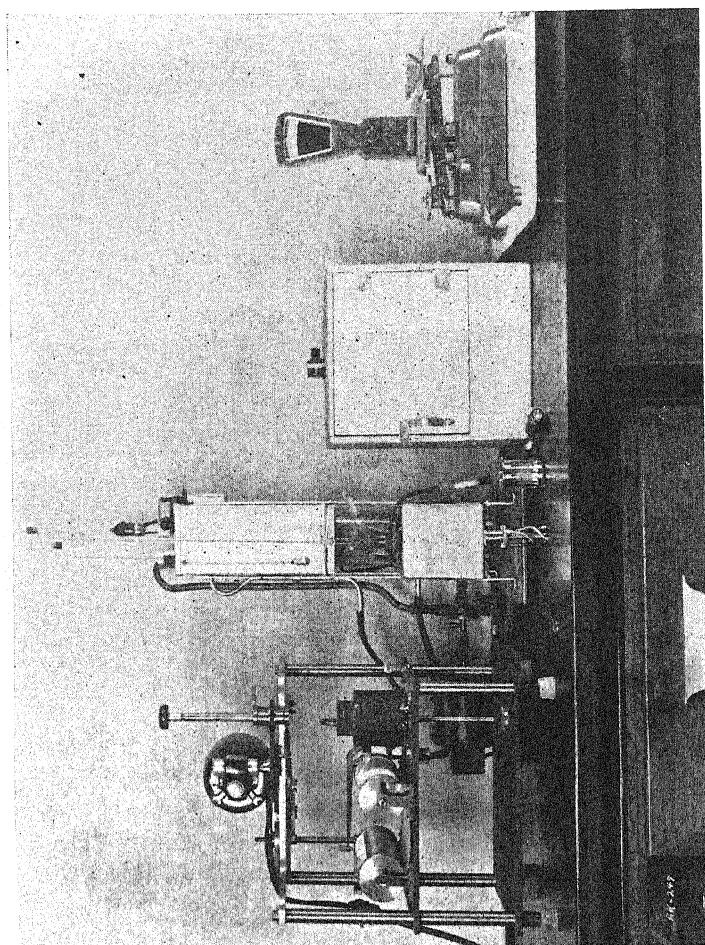


Figure 1. Recording mixer and auxiliary equipment.

(1) to keep the dough down to the bottom of the cup, and (2) to reduce the jerky action that intermittent passing of straight pins would produce.

In the model now in use the spindle was cut by hand from a stainless steel cylinder and the cup-pins were bent from stainless steel rod with the aid of a jig. The spindle can be reproduced accurately by

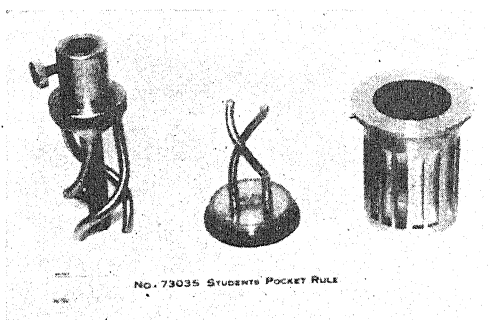


Figure 2. Spindle and cup.

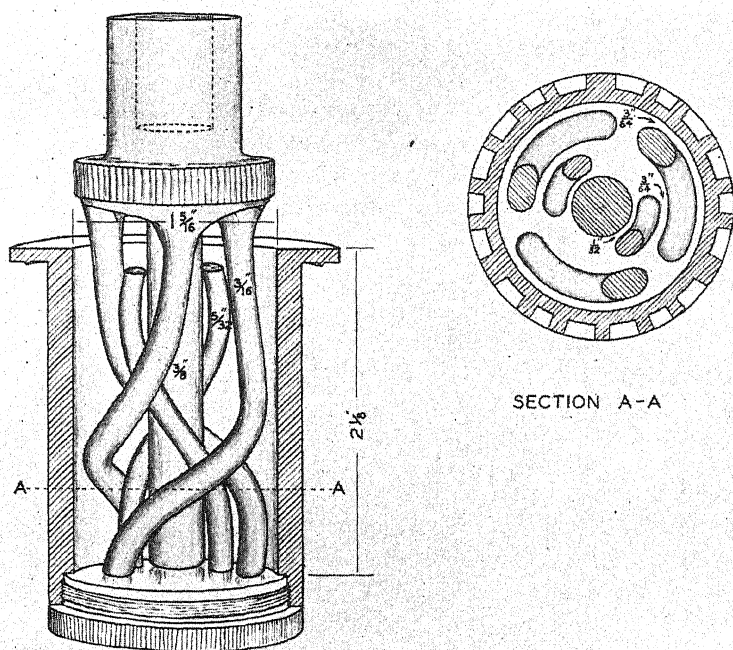


Figure 3. Spindle and cup assembly.

hand but difficulty has been experienced in bending the cup-pins to constant curvature. It is intended to modify slightly the shape of both the cup and the spindle pins to permit machine manufacture. The cup was machined from a solid piece of stainless steel and is provided with external ribs to improve the heat transfer to the jacketing water. To facilitate cleaning, it has a removable bottom machined to a water-tight fit.

The rotation of the cup is recorded by the movement of a pen arm on a kymograph roll driven at a paper speed of 0.44 mm. per second by a ratchet device from the motor. The torque, calculated as being exerted in the plane of the centres of the curved spindle pins, has a minimum value of 1225 g. and a maximum of 7500 g. with the present setting of the spring tension. These values may be raised or lowered, or the range between them increased or decreased, by altering the spring tension or by changing the springs, within the limits imposed by the power of the motor and the structural strength of the pins. With the present range, a pen movement of 1 mm. represents a change of 44.65 g. Plain paper strips are used for recording and the values read off by means of a scale scratched on celluloid. In the preliminary experiments the scale used was graduated in millimeters on both axes, but a change will be made to one reading directly in seconds and grams.

Figure 1 shows also the auxiliary equipment used in the tests: the scale for weighing the samples, a small constant temperature cabinet in which the samples are brought to mixing temperature ($30^{\circ}\text{C}.$), and the apparatus which supplies water at constant temperature to the cup jacket and also controls the temperature of the water used to mix the doughs.

Technique

The technique of the test is simple. Flour samples containing 7 g. dry matter are weighed out from larger samples stored in the tempering cabinet. The desired amount of water is run into the cup from the burette and the flour is poured in on top of the water. The cup is placed in position in the water jacket. The spindle is attached to the shaft and gently pushed down into position in the cup. The kymograph drive is turned a short distance by hand to mark a base line for measurements and the machine is started. At the end of the run the cup and spindle are removed, washed under a jet of water at approximately $30^{\circ}\text{C}.$, and dried with a clean towel. One man regularly makes about 8 single tests an hour including all operations except the measurement of the curves.

Character of Curves

The curves obtained differ in several respects and some of the types are shown in Figure 4. The height and time of occurrence of the maximum vary, as does the shape of the curve from the start of mixing to the maximum. There is a break in the curvature of the line some

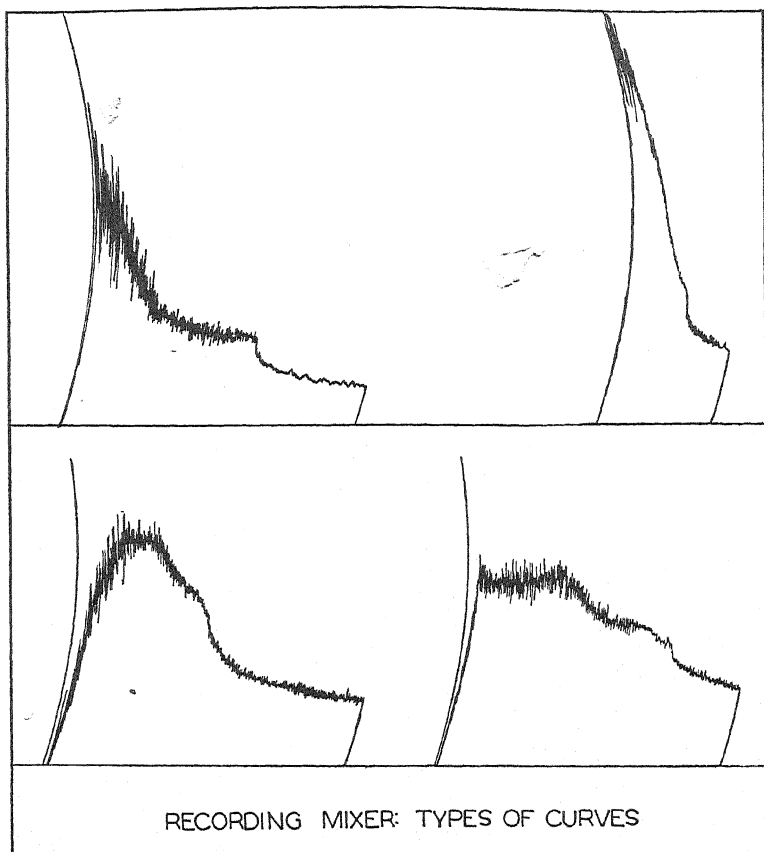


Figure 4. Types of curves.

time after the maximum, the position and sharpness of which are characteristic of different flours and treatments. The slopes and widths of line both before and after the break also vary. Each of these characters will have to be investigated in detail before the significance of the curves in terms of dough characteristics can be completely determined.

The curve can be quite accurately described by the height and time of the maximum, the height and time of the break, the angle at this point and by equations for the portions of the curve before and after the break. Equations for typical curves are shown in Table I.

TABLE I
EQUATIONS FOR PORTIONS OF CURVES BEFORE AND AFTER THE BREAK

Flour	Before break ¹	After break ²
A	$H = 1.46 T^{1.45}$	$T = .51 e^{.12 H}$
B	$H = 1.64 T^{1.30}$	$H = .53 T^{.46}$
C	$H = .8 + 1.1 T$	$T = 2.34 e^{.08 H}$
D	$H = -2.0 + 2 T$	$H = .57 T^{.48}$
E	$H = .17 T^{1.3}$	$T = 1.31 e^{.22 H}$
F	$H = 2.16 e^{.327 T}$	$T = .658 e^{.139 H}$
G	$H = 1.62 e^{.11 T}$	$H = .9 + 2.67 T - 0.84 T^2$
H	$H = 2.27 T^{1.40}$	$H = 1.82 T^{.43}$

¹ H = distance above the break; T = time before the break.

² H = distance below the break; T = time after the break.

Preliminary experiments have shown that the height of the maximum is affected by the total water in the dough (water in flour + water added) but that the moisture content of the flour and hence the amount of water added to bring the dough to constant water content also have an effect. It is probably affected also by the grade of the flour.

So far it has been possible to make a detailed study of only one feature of the curves, namely, the break. The position of the break is reproducible in replicate samples with a surprising degree of accuracy. Typical examples of the checks between triplicate determinations of the height and time of occurrence (measured in millimeters) are given in Table II, but a more reliable estimate of the reproducibility of these measurements can be obtained from the standard errors derived from 48 sets of triplicate determinations. These standard errors are 1.46 mm. for the height of the breaking point and 0.88 mm. for

TABLE II
REPLICABILITY OF POSITION OF BREAKING POINT

Flour	Height, mm.			Time, mm.		
	1	2	3	1	2	3
A	55	58	57	27	27	27
B	48	49	49	30	30	28
C	33	33	33	30	30	30
D	19	21	20	20	21	21
E	13	14	16	43	46	45
Standard Error (144 curves)	1.46 mm.			0.88 mm.		

the time of breaking. In other words, differences between the means of duplicate determinations are statistically significant if they exceed 3 mm. for height or 2 mm. for time of breakdown. As the height may vary from 10 mm. to 120 mm. and the time over about the same range, it is hardly possible that such a level of reproducibility could be obtained unless the measurements had some definite relation to dough character.

Significance of Height of Break

Figure 5 shows the enormous difference in height when two samples of flour with different protein contents are doughed with equal amounts of water, while Figure 6 shows the effect of varying the amount of

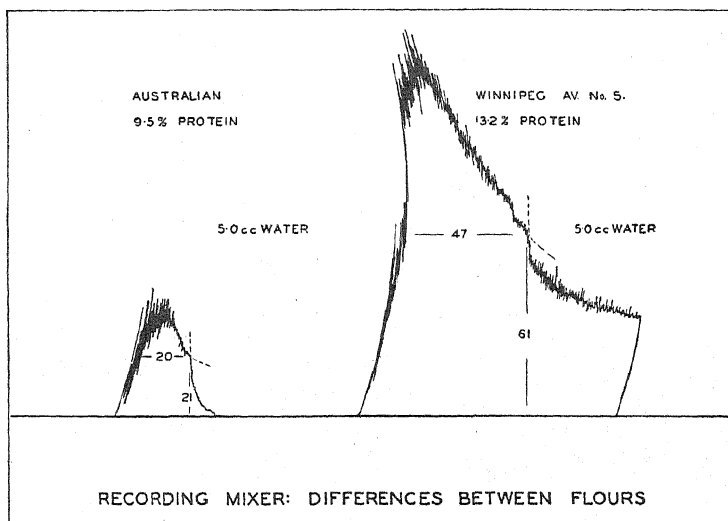


Figure 5. Curves from flours of different protein content.

water added. Other factors that probably influence the height of the break are: the grade of the flour, the granulation, the moisture content of the sample and the soundness of the wheat from which the flour was milled. So far, however, no direct experiments have been made on the effect of these factors.

The variation with protein content is important because it links one of the characteristics of the curves with a constituent of the flour. In a series of four varieties of wheat grown at seven different points in Alberta the simple correlation between protein content and the height of the break was $r = 0.71$ (1% point = 0.49) when the samples

were doughed with a constant amount of water, and an earlier series gave an even higher coefficient ($r = 0.88$, 1% point = 0.49).

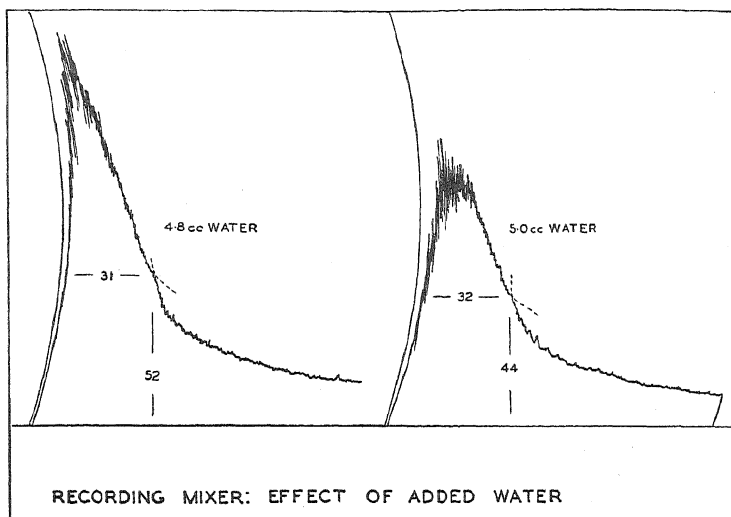


Figure 6. Effect of variation in amount of water added.

Significance of Sharpness of Break

In the Alberta series, mentioned above, it was noticed that there was considerable variation in the sharpness of the break. The angle between the slope of the lines before and after the break was measured for each curve so that increasing angle indicated increasing sharpness of break. This angle was correlated with protein content and the coefficient was found to be $r = 0.90$ (1% point = 0.49). As with the correlation reported in the last section, the importance of this lies in the establishment of a definite relation between the mixer curves and one of the factors of baking quality, rather than in the possibility of estimating protein content from the curves. The Kjeldahl determination is a simpler method of obtaining this information.

The sharpness of the break is influenced also by the amount of water added but except at the extremes of the range of the machine the change is comparatively small. In the Alberta series the average change in angle for an increase of 3% in absorption was only slightly over 2° while the range between flours was 50° .

Significance of Time of Occurrence of Break

Variations in the breaking time from about 35 seconds (16 mm.) to about 250 seconds (118 mm.) have been obtained with different

flours. The difference between two flours can be readily seen in Figure 5.

Where different amounts of water are added to the same flour (Figure 6) the breaking time is practically unaffected. This is shown for a number of flours in Table III. Curves were also obtained from 16 flours, to each of which three different amounts of water were added and the determinations made in triplicate giving nine results for each flour. Standard deviations were calculated: (1) regarding the nine curves from each flour's replicates in spite of the variations in water added and (2) in the ordinary manner from the triplicates. The former was 1.44 mm. and the latter 0.88 mm., indicating that the variation in water added made little difference to the breaking time. Later work has confirmed this conclusion.

TABLE III
EFFECT OF AMOUNT OF WATER ADDED ON BREAKING TIME

Water added	Breaking time, mm.				
	A	B	C	D	E
Control	42.3	26.7	40.0	58.7	18.7
+ 0.2 cc.	43.0	27.3	40.3	57.3	20.0
+ 0.4 cc.	43.3	29.7	40.3	56.0	20.3

Standard Error (144 curves)—at a single moisture 0.88 mm.
—neglecting moisture 1.44 mm.

This result was so striking and unexpected that further experiments were performed to test the constancy of the breaking time under different conditions. In the first experiment samples were diluted with 25% of starch without affecting the breaking time. Figure 7 is typical of the results obtained. Curves were also made with samples composed of starch mixed in various proportions with dried gluten.² The results, given in Table IV, again show the breaking time to be constant.

TABLE IV
EFFECT ON BREAKING TIME OF PROPORTION OF STARCH USED TO
DILUTE DRIED GLUTEN

Dilution		Breaking time, mm.						
Gluten, %	Starch, %	A	B	C	D	E	F	G
20	80	56	24	18	38	31	32	24
15	85	56	24	18	40	30	31	24
10	90	56	26	17	40	32	36	22

² These gluten samples were prepared by T. R. Aitken of the Laboratory of the Board of Grain Commissioners, Winnipeg.

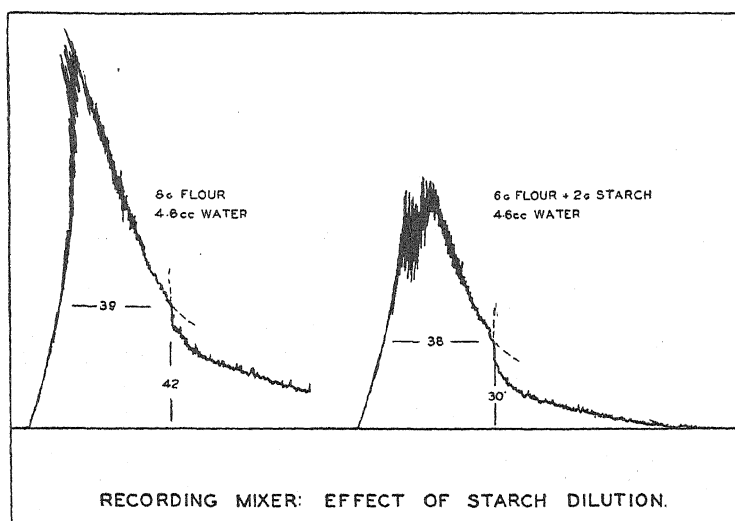


Figure 7. Effect of addition of starch.

Still another experiment was performed in which the weight of sample was varied from 7 g. to 9 g. (as received, moisture basis) and again the breaking time was unaffected (Table V).

TABLE V
EFFECT OF WEIGHT OF SAMPLE ON BREAKING TIME

Weight, g.	Breaking time, mm.		
	Flour A	Flour B	Flour C
7.0	34.5	42.0	37.0
7.5	34.5	42.0	36.0
8.0	36.5	41.5	38.0
8.5	36.0	42.5	39.5
9.0	—	43.5	38.5

It is apparent that the breaking time is characteristic of the flour. It is independent of the protein content and of the amount of water added except when excessive amounts of water are added or the protein content is abnormally low. If the protein content is reduced below a point not yet accurately determined but in the neighborhood of 7%, the breaking time decreases with increase in the water added.

To obtain preliminary information on the meaning of the breaking time in terms of flour characteristics, reagents known to affect colloidal properties were added to the doughs. Sodium chloride, lactic acid and ethyl alcohol were used and the results with one flour are shown in Figure 8. All of these reagents have a marked effect on the breaking

time, which therefore seems to be a measure of some colloidal property or properties. It is probably not a measure of the hydration capacity since lactic acid, which should increase hydration, and alcohol, which

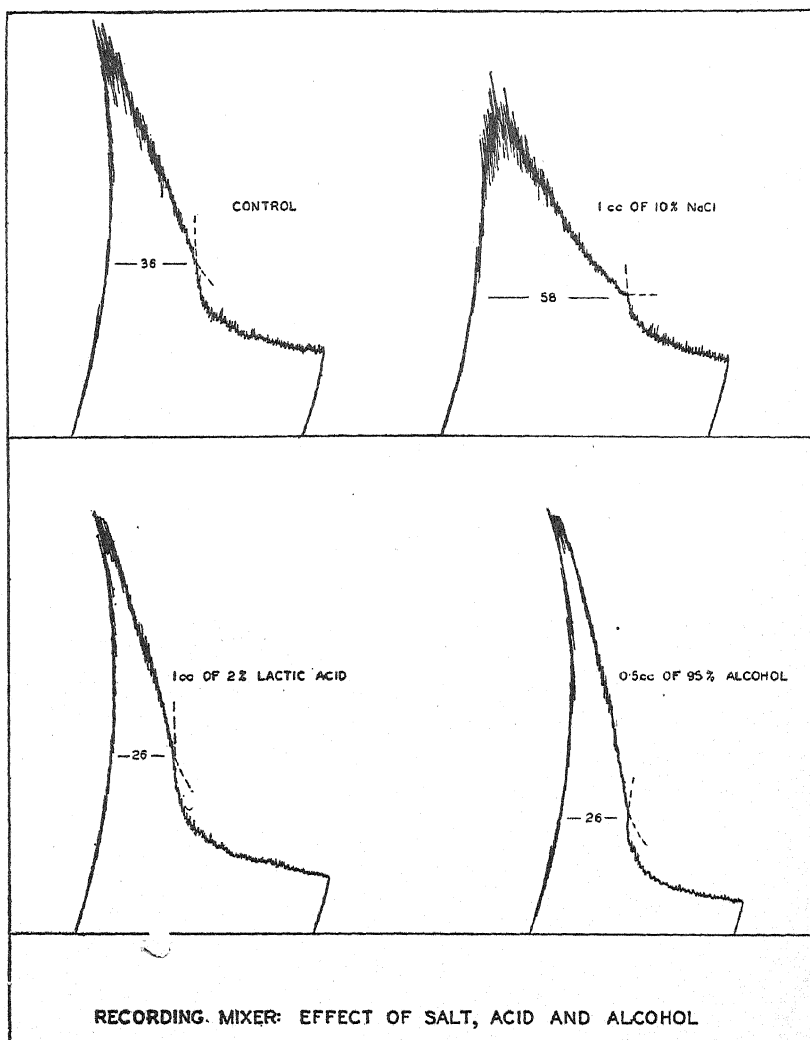


Figure 8. Effect of salt, acid and alcohol.

should reduce it, both decrease the breaking time. One might speculate that the break is indicative of a modification of the linkages of the gluten network, possibly accompanied by a change in the loosely-bound water.

The interpretation of the breaking time in terms of baking quality is a matter of some difficulty. Obviously, if a relation exists it must be through the gluten quality but as this term is generally used it includes not only the quality of the protein as it exists in the flour, but the modification of this quality by other dough ingredients and by factors operative during fermentation. So, even if there were a perfect relation between the breaking time of flour-and-water doughs and the quality of the gluten as present in the original flour, this quality might be so modified during fermentation that there would be no apparent relation between the breaking time and the gluten quality as inferred from the results of baking tests. The problem is also complicated by difficulties in the statistical analysis of pertinent data. Although it has been shown experimentally that protein content and breaking time are independent, it is difficult to obtain a series where they are not correlated, because of the effect of environment on both. This introduces complications in the application of the partial correlation method. Furthermore, protein quality can only be inferred from the results of baking tests and the inference may be greatly in error. Finally, it is unlikely that the breaking time is a complete measure of protein quality and any relation may be obscured by variation in quality due to unmeasured factors.

It is interesting that on a series of 28 samples a significant partial correlation coefficient ($r = -0.44$, 5% point = 0.38) was obtained between breaking time and loaf volume, holding protein content constant. There is some indication that the relation is markedly non-linear and therefore that it cannot be properly represented by such a coefficient. The evidence points to the existence of a relation between breaking time and gluten quality but this point obviously requires a great deal more investigation before it can be definitely established.

Use of Mixer with Fermenting Doughs, Gluten and Wheat

Fermenting doughs. The mixer operates satisfactorily with doughs of the consistency normally used in test baking, and changes in the dough during fermentation can be detected. For this purpose dough sufficient to provide the desired number of samples is mixed by hand or in a larger mixer. Aliquots are cut off from this bulk and placed in the recording mixer at the desired intervals. To avoid the possibility of the modification during one run affecting the characteristics at later runs, no sample of dough is used twice in the recording mixer. Figure 9 shows typical curves for fermenting dough and incidentally the effect of bromate on the particular flour. It should be noted that the breaking time is not immediately affected by the addition of bromate but differences appear as the fermentation proceeds.

There are striking differences between flours with respect to change in breaking time during fermentation. This is illustrated by the curves in Figure 10 in which the breaking time is plotted against the length of fermentation. It is interesting that these two flours gave very different relative results when tested on this continent by our American methods and when tested in Great Britain.

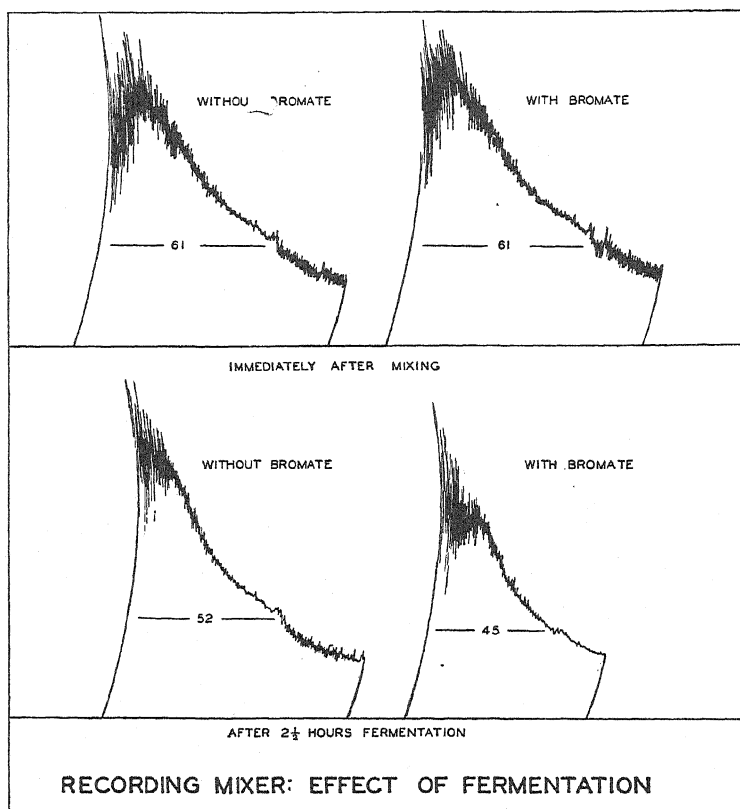


Figure 9. Curves from fermenting dough.

Gluten. The machine gives characteristic curves with both wet gluten and gluten dried by Aitken's method, provided it is suitably diluted with an inert substance such as starch or talc.

Ground wheat. In view of our original objective of providing a method of testing plant breeders' samples, it is of importance that the machine can be used with ground wheat and still more important that there is a close relation between the breaking time of the wheat and of the flour milled from it. In a paired series of 34 samples of wheat

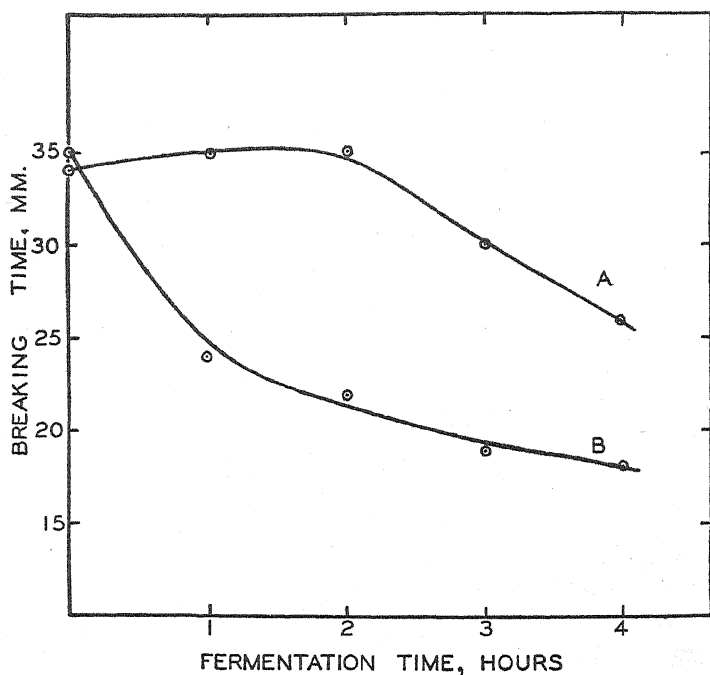


Figure 10. Effect of fermentation on breaking time.

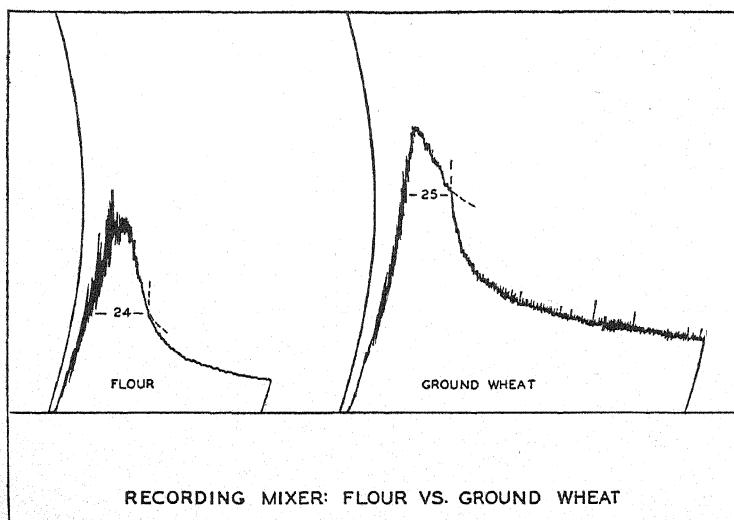


Figure 11. Comparison of curves from wheat and from the flour milled from it.

and 34 samples of flour, the simple correlation between the breaking time of the wheat and the breaking time of the flour was $r = .88$ (1% point = 0.45). Although this relation is not close enough to permit certain prediction, it is close enough for purposes of rough classification. Typical curves of ground wheat and flour from the same sample are shown in Figure 11.

Ground wheat shows the same constancy of breaking time as flour within a normal protein range. However, the protein content below which this constancy is lost is higher with wheat than with flour. With Canadian wheat the change may occur at about 11% (13.5% moisture basis).

Discussion

The experiments made so far have merely opened up the field sufficiently to show that the machine promises to be useful in cereal investigations and to show the lines along which further work may profitably be directed. If the apparent relations between curve characteristics and dough properties can be definitely established, the mixer will be a useful tool in many types of investigation besides the testing of plant breeders' samples, which inspired its construction. However, definite relations must be proven to exist, as the mere fact that flours of different quality give different curves is too general an observation for precise work. The use of such vague terms as dough development to cloak ignorance of the real meaning of curve characteristics is not a satisfying scientific device.

The investigations must proceed along two main lines: (1) attempts to relate curve characteristics to the composition or colloidal properties of the flour or dough, and (2) attempts to relate curve characteristics to baking quality, including dough quality. Neither line of investigation will be easy but a substantial body of information must be gathered if the interpretation of the curves is to rest on a sound foundation.

Summary

A new recording mixer which operates with flour samples containing 7 g. of dry matter is described.

The curves obtained with different flours show marked differences. There is a break in curvature in each curve and this break varies in position and sharpness.

The height and sharpness of the break are both related to the protein content of the sample.

The time at which the break occurs is independent of the protein content, the amount of water added, and the weight of the sample,

but is affected by the addition of reagents known to affect colloidal properties.

There are indications that the time of the break is related to gluten quality.

The mixer can be used to follow certain changes in fermenting doughs.

Curves can be obtained from wet or dried gluten diluted with starch or talc.

The breaking time of curves obtained with ground wheat is closely related to the time obtained from corresponding samples of flour.

Acknowledgment

The author wishes to acknowledge indebtedness to his colleagues on the Associate Committee on Grain Research for supplying him with samples and to his laboratory assistant, K. Hlynka, for his contribution to the constructional details of the mixer.

THE COLLOIDAL BEHAVIOR OF FLOUR DOUGHS. III. STUDIES UPON THE PROPERTIES OF FLOUR-STARCH-WATER SYSTEMS¹

MAX C. MARKLEY

Division of Agricultural Biochemistry, University Farm, St. Paul, Minnesota

(Read at the Annual Meeting, May 1937)

In the preceding sections of this study the properties of starch-water and flour-water systems under agitation in the bowl of a recording dough mixer were investigated (Markley 1937,² 1938³). The starch-water system was of a thixotropic nature with the maximum viscosity when at rest but with a decreasing viscosity when in motion. The flour-water systems were quite different; in them the viscosity tended to increase with mixing to a maximum, then to decrease. The principal factor differentiating flour from wheat starch is the gluten. Chemists and bakers have long assigned a role of major importance to the gluten in bread production. In the preceding paper it may be noted in Table I that the low-protein cake flour took less water and less time for development to the point of minimum mobility than did the high-protein spring wheat flours. However, it was not at all clear as to whether this observed difference in flours of diverse protein content was

¹ Paper No. 1523, Scientific Journal Series, Minnesota Agricultural Experiment Station.

² Markley, M. C. The colloidal behavior of flour doughs. I. The thixotropic nature of starch-water systems. *Cereal Chem.* 14: 434-436 (1937).

³ Markley, M. C., and Bailey, C. H. The colloidal behavior of flour doughs. II. A study of the effects of varying the flour-water ratio. *Cereal Chem.* 15: 317-326 (1938).

due to quantity, or to quality of the protein, or both. The investigation of this point was then attempted.

The effect of varying the quantity of protein while holding quality constant could be studied in either of two ways. One would be to concentrate the protein by a washing out of some starch, but this method has the disadvantage of being unsuited to dough formation studies. The other method is to dilute the flour with wheat starch. While there are some decided objections to this procedure, yet none appeared important enough to make it inadvisable, and accordingly three of the flours used in the preceding section were selected for this purpose. One was the high-protein spring-wheat straight-grade flour, the second the straight-grade flour milled from the semi-hard Minnesota winter wheat, while the third was the low-protein, soft, cake-type patent flour. The starch used in this study was confectioners' grade wheat starch graciously donated by the Huron Milling Company of Harbor Beach, Michigan.

The method of attack upon this problem was to make blends of the flours with the starch in all proportions from 100% flour to 100% starch. Doughs were mixed in the Farinograph in 300 grams of flour at 13.5% moisture with sufficient distilled water to reach a constant minimum mobility of 550 ± 20 Brabender units at 30°C . The resulting curves were measured for time to reach the point of minimum mobility. The data from this study are given in Table I.

The relation between the protein content of the flour-starch blends and the absorption required to bring the doughs to a constant minimum mobility is graphically shown in Figure 1. As the protein content of the blend is reduced from that of the natural flour the absorption tends to decrease, but when the protein has been lowered to about $7\frac{1}{2}\%$ there appear minima in the absorption, and as the protein content is further lowered below the $7\frac{1}{2}\%$ level the absorption begins to rise until the absorption of the 100% starch is about the same as that of the high-protein spring-wheat flour.

From these curves it is possible to differentiate between flours as to the relative water-holding powers of their proteins. To illustrate this the spring-wheat flour in its natural state at 14.3% protein required 69% of water to form the standard dough, but at its minimum at 7.3% protein required only 62.5%; while the soft flour of 7.9% protein required 54% in natural state and 53% at its minimum of 7.1% protein. The natural flours had a difference of 15% in their water-absorbing power, but after the flours had been brought to a corresponding protein level by the additions of starch there was still a difference of 9.5% in absorption which is about $\frac{2}{3}$ of the original difference. This probably indicates a greater water-binding power of the hard-

TABLE I
FLOUR-STARCH-WATER DOUGHS MIXED IN BRABENDER FARINOGRAPH AT MINIMUM
MOBILITY OF 550 BRABENDER UNITS

Flour	Starch	Protein	Absorption	Time to point of minimum mobility
<i>Flour No. 16245, High-protein Spring-wheat Straight</i>				
%	%	%	%	minutes
100	0	14.3	69.0	10.50
98.2	1.8	14.0	68.5	9.00
95	5.0	13.6	68.5	13.00
90	10.0	12.9	67.5	8.25
85	15.0	12.2	65.5	7.00
80	20.0	11.5	65.0	6.50
75	25.0	10.8	64.5	5.00
70	30.0	10.1	64.5	5.25
65	35.0	9.4	64.0	3.00
60	40.0	8.7	63.0	1.75
55	45.0	8.0	63.0	1.75
50	50.0	7.3	62.5	1.25
45	55.0	6.5	63.0	0.75
40	60.0	5.8	63.5	1.25
35	65.0	5.1	64.0	1.00
30	70.0	4.4	65.5	1.00
25	75.0	3.7	66.0	1.25
20	80.0	3.0	66.5	1.25
15	85.0	2.3	67.0	1.25
10	90.0	1.6	68.5	1.40
5	95.0	0.8	69.5	1.50
0	100.0	0.2	70.5	0.75
<i>Flour No. 16246, Minnesota Winter Straight</i>				
100	0	12.00	58.5	6.00
95	5	11.40	58.5	5.00
85	15	10.20	58.5	4.50
80	20	9.65	58.0	4.75
75	25	9.05	58.5	1.50
70	30	8.45	57.5	1.75
65	35	7.90	57.5	1.25
60	40	7.30	59.0	1.25
50	50	6.10	60.5	1.25
40	60	4.90	61.0	1.25
30	70	3.75	63.5	1.25
20	80	2.55	66.0	1.25
10	90	1.40	68.5	0.75
0	100	0.20	70.5	0.75
<i>Flour No. 15964, Soft Cake Patent</i>				
100	0	7.90	54.0	1.00
95	5	7.50	53.5	1.70
90	10	7.10	53.0	1.50
85	15	6.75	53.5	1.50
80	20	6.35	54.0	1.00
75	25	5.95	54.4	1.20
70	30	5.60	55.0	1.00
60	40	4.80	56.0	1.00
50	50	4.05	59.0	1.00
40	60	3.25	60.0	1.00
30	70	2.50	63.5	0.70
20	80	1.75	66.0	0.70
10	90	0.81	68.5	0.70
0	100	0.20	70.5	0.75

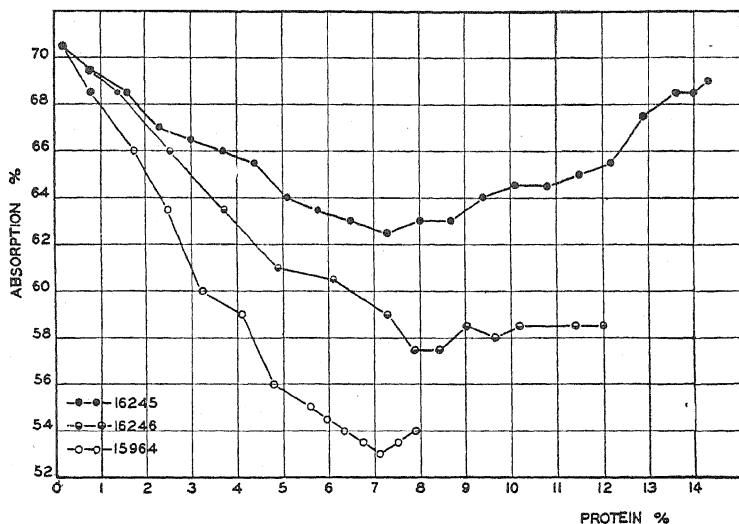


Figure 1. Relation between the protein content of flour-starch blends and the amount of water required to produce doughs of 550 Brabender units minimum mobility.

wheat flour as compared to the soft cake flour. It does not seem quite as probable that the starches involved would be sufficiently different in water-binding power to account for this difference, though such might be the reason.

The relation between the protein content of the flour-starch blends and the time required to bring the doughs to the point of minimum mobility is graphically shown in Figure 2. In this figure it may readily be seen that as the protein content is decreased the time required to develop the dough also decreases simultaneously until a protein level of 8% is reached. At protein levels below 8% the mixing time is constant at about one minute. No differentiation could be noted between the three flours used in this study.

This broken curve is of great interest. The characteristics of the individual Farinograph charts at protein levels below 8% were all very similar to the starch curve presented in Figure 1 of the first section of this study. At levels around 8% the curves from all flours were identical with very short mixing times, typical of the low-protein flours. As the protein content increased the development time likewise increased until the curves were like that in Figure 1 of the second section (Markley, 1938). The break-point in the protein-development time relation occurring at 8% protein corresponds quite well with the 7½% protein minimum for the protein-absorption relation. It would appear that at least 7% of protein must be present if there is to be a continuous gluten structure throughout the dough at mobility levels normal to

bread doughs. At the present time the question of gluten structure in cracker and macaroni doughs, and in cake batters is still unanswered. The more protein present above this minimum amount of 7% the higher the viscosity of the dough, primarily because of the attraction between the protein micelles. The first 7% of protein is apparently held in a much different manner in the system. The increasing water-holding capacity of the system as the protein content is reduced below

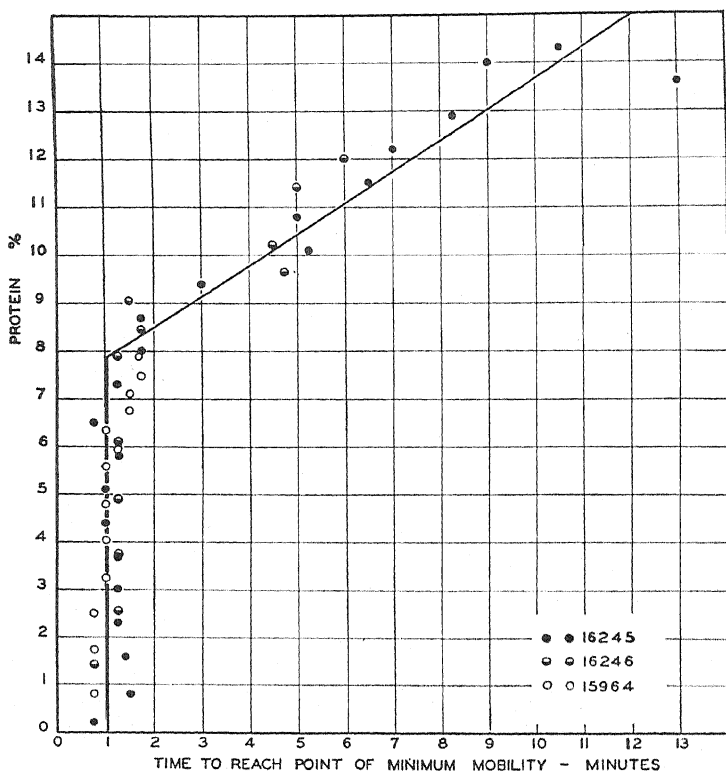


Figure 2. Relation between protein content of flour-starch blends and the time required to mix the doughs to a constant minimum mobility of 550 Brabender units.

7% can possibly be accounted for by postulating an attraction between the starch and the protein which would reduce the water-binding power of both. This may be a form similar to coacervation with all the charges on the protein micelles bound by the starch, and with the protein forming a film over the surface of the starch granules. Possibly 7% protein is just enough to coat the starch, and at this point there is a minimum of attraction between particles or for water. With increas-

ing amounts of protein above 7% there are more active charges upon the proteins than can be held by the starch, and as a result the starch granules are held together by interlocking protein micelles extending out from the protein films over the granules. More water is bound in such a system as well. When the protein content is reduced below 7% there is not enough protein to cover the surface of the starch and the formation of a rigid but fugitive starch gel is possible. This would account for the temporarily higher absorption of the low protein and starch doughs. Such a picture as this needs confirmation by other physical techniques before it is worthy of serious acceptance.

The viscosity of a starch-water paste is primarily a function of the ratio of the volume of the dispersed solid phase to the volume of water. The volume of the solid phase not only includes that of the starch granule itself, but also that of a shell of water more or less tightly bound to the surface of the granule by surface forces of one type or another. This shell can be removed by prolonged shearing stresses as in a dough mixer. The addition of protein up to 7% must reduce the particle size since less water is needed to maintain a constant maximum viscosity in the system. The reduction in particle size by the addition of protein up to the 7% level probably is due to the formation of a more or less complete film or envelope of protein over the surface of the starch granule, with a partial satisfaction of the surface forces by the starch to protein attraction, resulting in a smooth granule with but little water-shell and little attraction to similar particles. If all the starch granules were of a uniform size then the thickness of this film would be about one twenty-fifth of the radius of the granules, but since starch granules vary widely in size this is a rather meaningless dimension. When the starch granule is just covered by the protein film, then the surface forces of the starch must almost exactly balance those of the protein shell, with the result that at this protein-starch ratio the volume of the individual particles is at a minimum. If the granule is not completely covered then there is a partial water shell, held by the unsatisfied surface forces of the starch, with the result that the viscosity is intermediate between that of starch and that of 7%-protein flour. If more than 7% protein is present, then the protein shell would be thicker and more irregular with many exposed secondary valence forces. This would result in both particle to particle attraction and the binding of water to the particles. The viscosity of the dough would then increase in proportion to the amount of protein present above the 7% level.

An examination of bread crumb stained with Millon's reagent indicates that the starch granules are closely packed and are enveloped in a close-fitting envelope of protein. This can only be seen under quite

treatment. Standardized methods of analysis (A. O. A. C.) were used throughout the course of this study. Exceptions were the modified 72% sulfuric acid method of Peterson *et al.* (1932) for lignin and the phytin phosphorus procedure of Averill and King (1926). Sucrose was determined from its rotation (9.20%) and from the yield of Cu_2O after hydrolysis (9.33%). The average of the values so obtained is reported. For the determination of copper the Elvehjem-Lindow (1929) modified pyridine-thiocyanate method was used. Lipoid phosphorus represents that which is present in the ether extract or crude fat.

Results

The composition of the germ with respect to its organic constituents (see Table I) is reported on a moisture-free basis, the calculation resting upon a loss of 7.86% on heating under reduced pressure at 70° .

TABLE I
ORGANIC CONSTITUENTS OF RYE GERM

	%	%
Ether extract		13.23
Crude protein ($\text{N} \times 5.83$)		39.76
Nitrogen of alcohol-soluble proteins	0.50	
Albuminoid nitrogen	6.06	
Nitrogen not accounted for	0.25	
Carbohydrates		27.37
Sucrose	9.28	
Raffinose	2.15	
Starch (by diastase)	7.18	
Pentosans	8.76	
Lignin		6.82
Crude fiber		2.44

Proteins soluble in relatively strong alcohol (70%) constitute 7.3% of the whole. Similarly, for albuminoids this factor is 89. The organic bases probably account for the remainder, a situation already known to obtain, according to Power (1913), with at least one other of the Gramineae.

A total phosphorus content of 0.66% was found, of which approximately 18% has been accounted for in organic combination, *viz.*: phosphatides as lecithin 0.364% and phytin 0.39% ($\text{P} \times 3.55$).

The so-called "nitrogen-free extract" amounts to only 5.4%, which value is reduced by 7% of itself if the figure found for cellulose (0.38%) be deducted. But, because of the unsatisfactory state of the pertinent analytical procedure, some doubt exists as to the validity of the latter in spite of the fact that excellent check results were obtained. Be that as it may, however, the low order of magnitude of the former value quite properly may be construed as an indication that the analysis has been fairly complete within the scope of the limitations of investigations of this type.

The ash content of the moisture-free germ was found to be 4.97%, with water-insoluble constituents (52.7%) predominating over the water-soluble. The alkalinity of the latter, however, exceeds by a small amount that of the former, or 1.20 and 1.04 c.c. 0.1 *N* acid per gram of sample, respectively. The content of each inorganic constituent, calculated to the form indicated within the accompanying parenthesis (see Table II), is expressed both in relation to the original germ and the total ash and, for the purpose of determining the potential acid-base balance of the germ along the lines followed by Sherman and Gettler (1912), in terms of the equivalent volume of normal acid or base solution per 100 g. of substance.

TABLE II
INORGANIC CONSTITUENTS OF RYE GERM

Constituent	Concentration		Acid or base equivalent N/1 sol. per 100 g. C.c.
	% of germ	% of ash	
Iron (Fe_2O_3)	0.033	5.63	3.4
Aluminum	trace	—	—
Manganese (Mn_2O_3)	0.024	0.48	0.6
Calcium (CaO)	0.090	1.81	3.2
Magnesium (MgO)	0.530	10.66	26.6
Sodium (Na_2O)	0.130	2.61	4.0
Potassium (K_2O)	0.510	10.26	10.8
Copper (Cu)	0.00056	0.01	—
Zinc (Zn)	0.0166	0.33	—
Silica (SiO_2)	0.052	1.04	3.4
Phosphorus (P_2O_5)	1.511	30.40	44.0
Sulfur (SO_3)	1.075	21.62	27.0
Chlorine (Cl)	0.085	1.71	2.4

Discussion

In so far as the results disclosed by the three major literature citations in question (see Table III)—they have been taken from German sources and presumably have reference to rye produced in that country—may serve as the yardstick for evaluating an American-grown product, it appears that there are in this instance no very significant differences in respect to content of either total oxidizable organic nutrients or of inorganic constituents. Specifically, the observed crude fat content of 13.23% lies within the reported limits of 11.95 and 14.44%; it is practically the same as the calculated average for this group but higher than that (10.71%) of all like data heretofore reported by others. The crude protein value of 39.76%, although substantially the same as the all-average of 40.36%, is below the 41.05-minimum of Rubner (1916), itself less than the maximum content, or 46.31%, which Kling (1910) found.

TABLE III
COMPARATIVE DATA ON THE COMPOSITION OF RYE GERM

Constituent	Kling-Rubner-Kalning		All available		Wisconsin, %
	Limits, %	Average, %	Limits, %	Average, %	
Crude fat	11.95-14.44	12.93	7.30-14.44	10.71	13.23
Crude protein	41.05-46.31	44.03	25.85-44.74	40.36	39.76
Pentosans	7.32- 8.04	7.56	7.32- 8.04	7.56	8.76
Crude fiber	2.63- 3.94	3.28	1.83- 6.70	4.41	2.44
Nitrogen-free ex- tract	32.81-33.83	33.32	28.65-51.43	37.64	30.84
Ash	5.54- 6.76	6.05	4.43- 9.65	6.43	4.97

Almost three decades ago the first information on the pentosan content of the germ (8.04%) was announced. Credit for this belongs to Kling (1910). Within short intervals thereafter appeared the practically identical data (7.32%) of Rubner (1916) and Kalning (1917), the average value for the group then becoming 7.56%, one which is about one-seventh less than that found for the domestic product which is 8.76%. Kalning (1917), apparently with indifferent success, alone appears to have attempted a determination of the starch content of the germ. He reported a value of *ca* 6%, one which at least approximates the order of magnitude of that herein recorded, or 7.18%. These findings, therefore, seem to invalidate the statement of Kling (1910) that rye germ contains no starch.

Because of the nature of the determination itself, one leading to results which, in a sense, are defined by the technique followed, it is perhaps to be expected that wide variations in the crude fiber content of an agricultural product will be reported. The rye germ is no exception for witness the minimum of 1.83%, a 3.7-fold maximum and an average—nine reports are involved—of 4.41%. Kling's (1910) value of 2.63% alone in this group compares with that reported herein, or 2.44%. The cellulose content of 3.13% which Rubner (1916) reported might well be accepted with some reservations for reasons above noted. It is eight times larger than that herein reported.

Sugar determinations have been attempted by Kalning (1917) but, unfortunately, his results, which were reported as reducing sugars before hydrolysis 6.66% and sugar after inversion 22.62%, are not very informative. Kling (1910) dismissed this phase of his investigation with the observation that the nitrogen-free extract (32.81%) may be deemed to consist practically all of carbohydrates. Frankfurt (1896) indicated that the sucrose, raffinose and glucose content of wheat germ totals 24.34% and later Power and Salway (1913) isolated the first two

from this source. Our own investigations have shown that rye germ contains 9.28% sucrose and 2.15% raffinose.

The hitherto published lower and upper limits of the mineral matter of this germ, as revealed in eleven communications, are 4.43 and 9.65%—the average of them all is 6.43%; for those reported by the three investigators cited it is 6.05%. The majority of the indicated values, however, are of the order of magnitude (4.97%) herein reported.

If anyone before has quantitatively examined the nature of the inorganic constituents of the rye germ, then this fact has escaped notice in a meticulous search of the literature on the subject. The results of such an analysis calculated to a dry basis are presented in Table II. They reveal the fact that rye germ is rich in phosphorus (less so in this respect than is wheat germ), sulfur, magnesium and potassium. Whether the apparently high content of iron and magnesium might not be in part due to an incidental contamination in the milling process is a matter for conjecture. The copper content of the germ is approximately that of white bread, Graham flour, dried dates and figs, oatmeal, sweet potatoes, and sweet Bantam corn (Lindow *et al.*, 1929) but less than that of the wheat germ which Elvehjem and Hart (1929) found to contain 9 mg. per kilogram. An interpretation of these data from the standpoint of potential acid-base balance by the Sherman-Gettler (1912) calculation reveals a preponderance of acid-forming elements. Although it appears that phosphorus (0.66%) is the most prominent element in this group, yet its concentration in the wheat germ is apparently greater. Andrews and Bailey (1932) found that the phosphorus content of wheat germ is 1.244%, that that in phytic combination constitutes 47.98% of the whole, and that in lipid form 5.70. Comparable data for rye germ were found to be 16.6 and 22.7% respectively.

There remains to be made, finally, comparison of the proximate composition of rye germ as herein reported with similar data on wheat and corn germ as gleaned from the literature and collated in Table IV.

TABLE IV
PROXIMATE COMPOSITION OF THE GERMS OF THE GRAMINEAE

	Wheat	Corn	Rye
Protein	23.60–40.75 ^{21 1}	11.98–15.50 ⁴	25.85–44.74 ¹¹
Fat	6.01–13.51 ²²	3.72–24.36 ⁴	7.30–14.44 ¹¹
Crude fiber	1.63– 4.75 ¹⁶	4.98– 5.76 ³	1.83– 6.70 ⁹
Starch	9.95–13.50 ³	24.75–47.50 ²	6.00 ¹
Pentosans	10.92 ¹	—	7.32– 8.04 ³
Ash	3.90– 6.40 ²⁰	2.77– 6.05 ⁴	4.43– 9.65 ¹¹
Nitrogen-free extract	39.25–48.50 ¹⁴	45.42–48.72 ³	28.65–51.43 ¹⁰

¹ Superior numbers indicate the number of pertinent samples.

This compilation is presented without literature citation for reasons of economy of space. It should be read with reference to the last column in Table III.

Summary

Rye germ is a high-protein, phosphorus-rich substance in which the lipid predominate over the phytin forms of the latter. In Germany it has received some measure of study as to its suitability for animal feeds. The opinion there expressed is that farm animals relish it and assimilate it readily. Incidental to such investigations there have been made studies in European laboratories on composition which, although doubtless extensive enough to serve the immediate objectives then in hand, admit of elaboration. A study of this character has been made on an American-grown product. No very significant differences in respect to content of either total oxidizable organic nutrients or inorganic constituents have been found. The so-called nitrogen-free extract has been broken down to the point where there remains *ca* 5% to be accounted for. A quantitative examination of the ash has shown that acid-forming elements predominate over the base-formers.

Literature Cited

- Andrews, J. S., and Bailey, C. H.
1932 Distribution of organic phosphorus in wheat. *Ind. Eng. Chem.* **24**: 80-82.
- Averill, H. P., and King, C. G.
1926 The phytin content of foodstuffs. *J. Am. Chem. Soc.* **48**: 724-728.
- Elvehjem, C. A., and Hart, E. B.
1929 The copper content of feedings stuffs. *J. Biol. Chem.* **82**: 473-477.
- and Lindow, C. W.
1929 The determination of copper in biological materials. *Ibid.* **81**: 435-443.
- Frankfurt, S.
1896 Zur Kenntniss der Zusammensetzung des ruhenden Keimes. *Landw. Vers. Sta.* **47**: 449-470.
- Kalning, H.
1917 Die chemische Zusammensetzung von Weizen-, Roggen- und Maiskeimen. *Z. ges. Getreidew.* **9**: 167; *Biedermanns Zentr.* **47**: 114-116.
- Kling, M.
1910 Roggenkeime. *Landw. Vers. Sta.* **72**: 427-435.
- Lindow, C. W., Elvehjem, C. A., and Peterson, W. H.
1929 The copper content of plant and animal foods. *J. Biol. Chem.* **82**: 465-471.
- Peterson, C. J., Walde, A. W., and Hixon, R. M.
1932 Effect of temperature on sulfuric acid method for lignin. *Ind. Eng. Chem., Anal. Ed.* **4**: 216-217.
- Power, F. B., and Salway, A. H.
1913 Chemical examination of wheat germ. *Pharm. J.* **91**: 117-120.
- Rubner, M.
1916 Ueber die Zusammensetzung und Verdaulichkeit der Keime einiger Zerealien. *Arch. Anat. Physiol. Abt.* **1916**: 123-131.
- Sherman, H. C., and Gettler, A. O.
1912 The balance of acid-forming and base-forming elements in foods and its relation to ammonia metabolism. *J. Biol. Chem.* **11**: 323-338.

A STUDY OF THE BACTERIAL POPULATION OF GRAINS USED IN A DISTILLERY

C. S. BORUFF, RALPH I. CLAASSEN, and A. L. SOTIER ¹

Hiram Walker & Sons, Inc., Peoria, Illinois

(Received for publication March 17, 1938)

Introduction

In preparing grain mash in a distillery, effective sterilization by heat is impossible both for economical and operative reasons. Corn is usually pressure-cooked but rye is normally mashed at 160° F. Malting or starch conversion operations are carried out at temperatures below 160° F. so as not to impair the desired enzymatic activity of the malt. Therefore, if a low bacterial count of the mash is to be accomplished, a careful check of the grains used must be made and those grains showing a high count must be rejected.

There are two objections to a high bacterial count in the mash. First, even a moderate number of bacteria in the original mash means there will be a much larger number present after one or two days of fermentation at 75° F. to 90° F. (24° C. to 32° C.). These organisms will grow at the expense of some of the carbohydrate present, thereby lowering the yield of alcohol. Second, while the yield of alcohol is being lowered, objectionable bacterial fermentation products are being formed and these adversely affect the quality of the distillate. Those by-products which are steam distilled with alcohol, and hence become a part of the whiskey, are especially objectionable.

Methods

Hoffman, Schweitzer, and Dalby (1937) have contributed a method of counting spores responsible for "rope" in bread, but their technique is not suited for the determination of total bacterial counts in grains.

Brahm (1921) contributed studies on the bacterial flora of flour and was probably the first investigator to propose bacteriological standards for this commodity. He proposed a limit of 150 "rope" spores per gram of flour for bakery use.

Since there is no standard or official method for the estimation of numbers of bacteria in grain, it was necessary to work out a practical method that would combine accuracy, speed, and ease of operation.

In determining the bacterial count of grains, important points to be observed are proper sampling, the avoidance of contamination in

¹ Technical Director, Development Supervisor, and Bacteriologist, respectively.

grinding and handling, the sterility of all glassware, diluting water and media, proper incubation, and lastly, proper counting.

Sampling. A probe sampler is used which collects 10 individual samples at one time. Five thrusts are made with this sampler at different points in the car of grain and the fifty individual samples composited in a clean, dry container. This is the standard procedure used to collect samples for moisture, damage, and other standard and special grain tests. After thorough mixing, the large sample is reduced by halving until about 50 g. remain.

Grinding. The 50-g. sample is next ground to a fine flour in a clean, dry mill. A No. 1 Wiley Mill using the 1 mm. screen is used for this purpose.

Numerous screen passage determinations have shown that this grinding gives a meal fine enough so that 95 to 97½% will pass a 30-mesh screen, with less than 1% being retained on a 20-mesh screen.

Sterilization of glassware and media. All glassware and media are prepared and sterilized in accordance with the recommendations of "Standard Methods for the Examination of Water and Sewage" of the American Public Health Association (1928). Difco Nutrient Agar is the medium used for plating.

Water blanks. In the Hiram Walker laboratory an effort has been made to reduce diluting transfers to a minimum, and for this reason, only 99 c.c. water blanks are used. These are prepared by filling 250 c.c. capacity round bottles with approximately 103 c.c. of water so that 99 c.c. remain after sterilization.

Rubber stoppers containing two inches of glass rodding are used instead of cotton plugs. The glass rod acts as a mechanical agitator during shaking and extends into the bottle when the stopper is in place.

After the dilution bottles have been filled with the required amount of water they are packed loosely in the autoclave without the stoppers in place. The stoppers are either placed on end or in a wire basket and sterilized along with the dilution water. Sterilization is for 20 minutes at 15 pounds pressure.

After the pressure in the autoclave has been released, the stoppers are immediately inserted into the water blank bottles. This operation is carried out within the autoclave. After the bottles are removed from the autoclave, they are allowed to cool before use.

Plating. Two dilutions can normally be run, namely, 1 to 100, and 1 to 10,000. One gram of the ground grain meal is weighed out on a balance and introduced into a 99 c.c. water blank. The stopper is not touched where it will come in contact with the inside of the bottle. During the introduction of the meal, the stopper is rested on the desk with the glass rod straight up, and after the introduction of the meal it is immediately replaced.

The bottle is then shaken vigorously for 20 seconds timed with a watch to obtain an even suspension of the finely ground meal and micro-organisms. If the grain is known to be of a low bacterial count, this 1 to 100 dilution is all that need be made.

The next higher dilution is made by withdrawing one cubic centimeter from the first dilution with a sterile pipette and introducing it into a second 99 c.c. water blank. This 1 to 10,000 dilution is sufficiently high for most grains. This second dilution bottle, like the first, is then shaken for 20 seconds in order to obtain an even suspension of organisms.

Duplicate petri-plates are prepared from the dilution selected by withdrawing one cubic centimeter of the suspension and introducing it in the center of the plate by means of a sterile pipette. Melted nutrient agar is next added, and with a swirling motion, the sample and agar mixed, the plate covered, and then left to harden. Incubation is for 24 hours at $37\frac{1}{2}^{\circ}$ C.

The counting of the plate after incubation is done in the usual way with the aid of an illuminated counting apparatus.

Accuracy of the method. The accuracy of any method cannot be proved until a number of workers in different laboratories have compared results of the method. The comparisons that have been made to date on this method indicate results that check within 10% (plus or minus) in grains containing approximately three million bacteria per gram.

Grain Counts

Bacterial count studies have been made of all grains used in the distillery. In Table I there are listed the monthly averages of the four

TABLE I
MONTHLY AVERAGES OF GRAIN COUNTS, OCTOBER 1936 TO MAY 1937
Total Bacteria Per Gram

Date	Barley malt	Rye malt	Rye	Corn
1936 October	2,400,000	—	800,000	15,000
November	2,800,000	1,000,000	900,000	35,000
December	2,700,000	3,600,000	1,200,000	2,400,000
1937 January	5,200,000	3,800,000	1,000,000	2,000,000
February	4,500,000	—	1,400,000	300,000
March	3,800,000	3,900,000	1,700,000	3,500,000
April	2,800,000	3,300,000	1,100,000	1,500,000
May	3,400,000	1,900,000	1,600,000	900,000
June	3,900,000	2,600,000	800,000	200,000

grains used from October 1936 to May 1937. Table II lists the maximum, minimum, and average count data of the four grains for the month of June 1937.

TABLE II
MAXIMUM, MINIMUM, AND AVERAGE COUNTS OF GRAINS SAMPLED IN JUNE 1937
Total Bacterial Count Per Gram

	Maximum count	Minimum count	Average count for month
Barley malt	7,700,000	700,000	3,900,000
Rye malt	4,600,000	1,600,000	2,600,000
Rye	1,700,000	60,000	770,000
Corn	800,000	30,000	240,000

From these tables it will be seen that of the four grains used, corn has the widest variation in its bacterial count. During October and November 1936, corn of the 1935 harvest was being used which had an average moisture content of only 13% and low bacterial count. During December a mixture of new-crop kiln-dried and new-crop natural-dried corn was used. It is noted that this corn showed a much higher average bacterial count. In February only kiln-dried corn of a low count was used. During March, April, and May, natural-dried corn of the 1936 crop was used. The moisture content of this corn averaged 15.9, 17.1, and 15.8%, respectively, for the three months. Increased bacterial counts are noted for this natural-dried corn over the kiln-dried corn used exclusively during February. This wide fluctuation in bacterial count is due to the type of drying the corn receives and its final moisture content, as well as the number of damaged kernels present.

Studies of the bacterial count of kiln- and natural-dried corn have been made and the averages of several determinations are given in Table III.

TABLE III
EFFECT OF KILN DRYING ON BACTERIAL COUNTS OF CORN

Type of drying	Number of samples	Bacterial count per gram	Moisture %
Natural dried	20	3,725,000	17.3
Kiln dried	12	990,000	14.5

The heat treatment necessary for kiln drying not only reduces the moisture content of the corn but markedly reduces the bacterial count. In the samples shown in Table III, this reduction in count amounts to about 400%.

Samples of damaged and undamaged corn kernels have been assayed for bacterial population. The results show that counts on damaged kernels run as much as 285 times greater than those of the

undamaged kernels. The data from typical studies are given in Table IV.

TABLE IV
EFFECT OF DAMAGED KERNELS ON BACTERIAL COUNTS OF CORN

Material	Bacterial count per gram	Ratio of counts on damaged and damage-free kernels
Damaged kernels	18,560,000	1 to 285
Damage-free kernels	40,000	
Damaged kernels	6,464,000	1 to 139
Damage-free kernels	45,000	

The malts have by far the highest bacterial count of the four grains used in a distillery. This is not surprising when one considers the nature of their preparation, for at no time after steeping and germination do they receive a heat treatment above 125° F. (52° C.). Studies in this laboratory have indicated that relatively few organisms are to be found on the outside of the kernels and that the bulk of the bacteria is to be found immediately under the outer covering of the kernel. Private communications from another laboratory have confirmed this finding.

As a result of studies made on the bacterial count of grains and the effect these bacteria have on yields and quality of the final distillate, bacteriological standards have been adopted for all grains used in the Hiram Walker Distillery. The maximum acceptable counts are given in Table V.

TABLE V
MAXIMUM ACCEPTABLE BACTERIAL COUNTS PER GRAM

Material	Maximum acceptable bacterial count per gram
Barley malt	10,000,000
Rye malt	10,000,000
Rye	3,000,000
Corn	3,000,000

It is interesting to note the reductions in bacterial counts that have followed these efforts. Before this work was started, counts as high as 30 to 50 million per gram were commonly found in barley malt. When these results were brought to the attention of maltsters, many cooperated fully in efforts to reduce the count in their products, with the result that malt is now regularly supplied by some maltsters having a count of only one tenth, or even less, than was formerly the case.

Summary

A method for counting bacteria in grain has been developed which is accurate, fast, and simple. Other laboratories using this method have obtained satisfactory checks.

The method is quite flexible, being applicable to grains of all sorts, husks of grains, flour, and related materials.

The development of the method has permitted studies of the bacterial population of grains and its effect on yields and quality of final distillates. These have resulted in the establishment of bacteriological standards for all grains used.

Literature Cited

- American Public Health Association
1928 Standard Methods for the Examination of Water and Sewage. Sixth Edition. Published by A.P.H.A., 370-7th Ave., New York.
- Brahm, C.
1921 New knowledge in rony bread and the organisms which induce this decomposition. *Z. ges. Getreidew.* **13**: 105-113.
- Hoffman, Charles, Schweitzer, T. Robert, and Dalby, Gaston
1937 Control of rope in bread. *Ind. Eng. Chem.* **29**: 464-467.

COMPARATIVE RATES OF AMYLASE ACTION ON STARCHES ¹

VERA DAWSON MARTIN and J. MARSHALL NEWTON

Department of Chemistry, Iowa State College, Ames, Iowa

(Received for publication March 17, 1938)

Several investigators (Stone, 1896; O'Sullivan, 1904; Ford, 1904; Nagao, 1911; Sherman, Walker, and Caldwell, 1919; Hermano and Rask, 1926) have reported differences in the rates of amylase action on starches, depending on the origin of the starch. Glock (1936) points out that the results are contradictory and suggests that each investigator may have been measuring a different effect of amylase action. The majority of investigators report potato starch to be more rapidly digested than the cereal starches. In this laboratory, while attempting to ascertain the lowest possible temperature for gelatinization of potato starch pastes for enzyme action, it was found that when the starch was heated at 70° C. for 30 minutes hydrolysis by soybean amylase was much faster than when the starch was boiled. This suggested that the discrepancies in the literature might be due to differences in methods of preparing the starch substrates. Day (1908) studied the effect of cooking different starches on their digestibility by amylase. Potato and arrowroot starch pastes were found to be

¹ Journal Paper No. J543 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 517.

digested as well if made with hot water and not boiled as those which had been boiled three hours. Corn and wheat starches were made somewhat more digestible by long cooking.

The methods and data reported here are a part of a detailed study of the effect of temperature of gelatinization on rates of hydrolysis of five different starches by soybean amylase. The starches used were corn, potato, rice, wheat and tapioca. Substrates were made from these starches by heating at 60°, 70°, 80°, 90°, 100° and 120° C. The rate of hydrolysis of the starch substrates by soybean amylase was measured by a potentiometric determination of the reducing sugars formed.

Preparation of the Starch Substrates

All substrates contained 2% starch and were at pH 5 as measured by the glass electrode. Ten grams of the untreated starch were stirred with 100 c.c. of cold water. This paste was poured into 350 c.c. of a solution containing distilled water and 49.0 c.c. of 0.2M NaH_2PO_4 solution and 1.0 c.c. of 0.2M Na_2HPO_4 , which had been brought to the desired temperature in a water bath. The mixture was kept at the desired temperature 30 minutes. It was then cooled to 40° C. and made up to 500 c.c. The substrates heated at 120° C. were prepared by boiling the mixture prepared as the others and then heating under 15 pounds of steam pressure in an autoclave for 30 minutes. The substrate was divided into two 250 c.c. portions and the same amount of enzyme added to each. The digestions were carried out at 40°. Five cubic centimeter portions were removed from each digestion at the same time for the sugar determinations, giving two values for every point on the curves.

Preparation and Activity of the Enzyme

The enzyme used was prepared from ether-extracted soybean meal by extraction with 50% alcohol and precipitation by adding absolute alcohol to make the concentration 70%. The dried powder had a saccharogenic power of 825 and zero dextrinogenic activity (see Ohlsson and Edfeldt, 1933). For each 250 c.c. of the 2% starch, 20 c.c. of a suspension in water containing 40 mg. of enzyme per 100 c.c. were used.

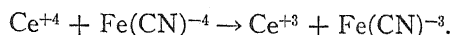
The ratio of enzyme to starch was one to 625, and the ratio of enzyme to maltose at 70% digestion was about one to 400. There was an excess of enzyme present at all times during the digestions.

Determination of Reducing Power of the Digestion Mixtures

It was not possible to use the modified Hagedorn and Jensen method for determining reducing sugars (see Blish and Sandstedt,

1933; Gore and Steele, 1935) to follow the course of these digestions. In the cases where the starch was heated at 60°, for instance, there were particles of unswollen starch in the digestion. These particles adsorbed the iodine and held it so tenaciously that it was impossible to duplicate results. The method of Hassid (1937) was modified to a macro determination, and the titration was followed potentiometrically.²

Five cubic centimeters of sample containing from 5 to 60 mg. of maltose were pipetted into 25 c.c. of alkaline ferricyanide reagent.³ This mixture was placed in a boiling water bath for 15 minutes, then cooled in running water 2 or 3 minutes. Twenty-five cubic centimeters of a 1-4 solution of HCl were added immediately, and the contents of the flask poured into a 250 c.c. beaker for titrating. The flask was rinsed into the beaker with two 10 c.c. portions of distilled water. The final volume of the solution was 80-85 c.c., and the acid concentration 1.0-1.5N. According to Furman and Evans (1929) this is the optimum acid concentration for the following reaction to proceed rapidly and quantitatively.



The solution was titrated potentiometrically with 0.1N ceric sulfate³ solution (see Furman and Evans, 1929) using a platinum-saturated calomel electrode system and a KCl-agar bridge. These electrodes were found to give an increase of 350 millivolts at the end-point in 1-1.5N acid. This voltage jump was much greater than could be obtained with a platinum-tungsten bimetallic electrode (see Furman and Wilson, 1928). Before titrating the solutions were green, and the color changed abruptly to yellow about 0.05 c.c. before the voltage change.

The results of these determinations were calculated to milligrams of maltose by converting the titration value to cubic centimeters of 0.1N ceric sulfate and reading the value for maltose directly from a graph prepared from data obtained by titrating solutions of known concentrations of C.P. maltose hydrate. The purity of the maltose was checked by the standard Munsen-Walker method. The results of the sugar determinations run on portions of the simultaneous duplicate digestions checked within 0.5 mg. The results on a repetition of the experiment showed a variation which was never more than

² The voltages were measured on an experimental model of an instrument operating directly off the 110 volt A.C. line, which can also be used to measure pH with glass or quinhydrone electrodes. The instrument is being manufactured for sale by Precision Scientific Co., Chicago, Illinois.

³ The reagents were prepared as follows: alkaline ferricyanide reagent—0.1N potassium ferrocyanide (by weighing) in 5% sodium carbonate solution; 0.1N ceric sulfate—53 g. of C.P. ceric sulfate (1.6 times the theoretical) were added to 900 c.c. of a solution containing 100 c.c. of concentrated sulfuric acid. This was digested on a hot plate until all the solid had dissolved. It was then filtered and made up to one liter. The solution was 1.0N in sulfuric acid. The ceric sulfate solution was standardized potentiometrically against a standard ferrous iron solution.

1% on a single determination. The time-maltose curves could be duplicated, therefore, with occasional points about 1% off the curve.

Data and Discussion of the Results

There was very little increase in the reducing power during the digestion of the various starches that had been heated at 60° for

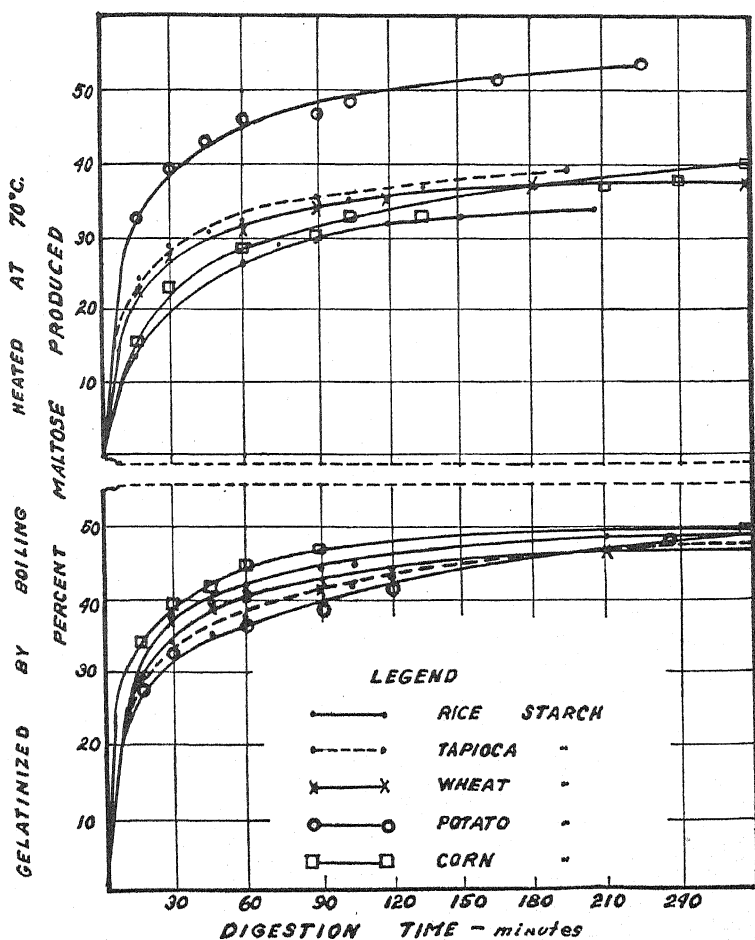


Figure 1.

30 minutes, except in the case of potato starch. Soybean amylase does not digest potato starch heated at 50°. Figure 1 shows the results of the sugar determinations plotted against time of amylase

action for starches which had been heated at 70° and 100° as previously described.

When the starches were prepared by heating at 70° C. for 30 minutes, the potato starch is digested most rapidly by soybean amylase. As shown in Figure 1, the tapioca starch is next in order and wheat, corn and rice are slower than either potato or tapioca. The limit of hydrolysis after 24 hours' digestion of the potato and tapioca starches heated at 70° was between 66 and 68% of the oven-dry starch. The limit for the cereal starches heated at 70° was 49 to 50%.

When the substrates were prepared by heating the starches at 100° for 30 minutes the order of rapidity of enzyme digestion is reversed. Corn starch was digested most rapidly, with wheat, rice, potato, and tapioca less rapidly, in order. The limit of digestion of wheat, rice, and tapioca starches heated at 100° was 57 to 60%, for corn starch 70% and potato starch 65% after 24-hour digestion. Heating the starches at a higher temperature slowed the enzyme action on potato and tapioca starches so much that the cereal starches were hydrolyzed more rapidly.

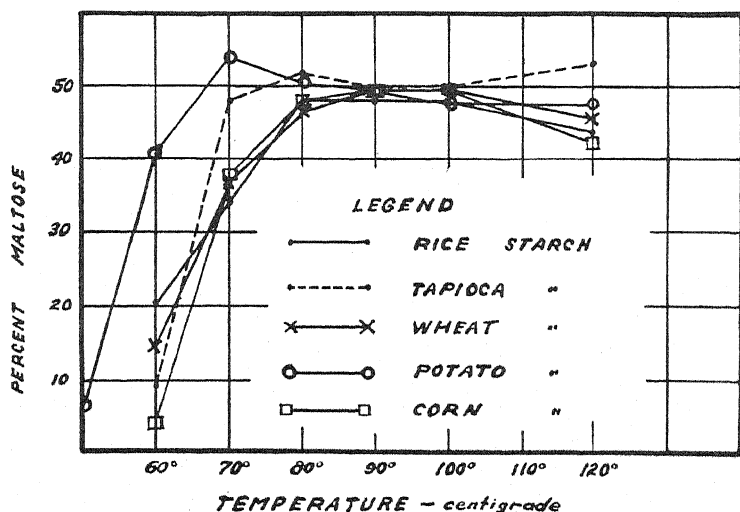


Figure 2.

The effect of heating the starches on the amylase action is shown more clearly by Figure 2 in which the maltose formed in 240 minutes of enzyme action is plotted against the temperature at which the starches were prepared. In every case except rice starch an optimum temperature of preparing the starch for soybean amylase action is indicated—70° for potato, 80° for tapioca, and 90° for wheat and

corn starches. Temperatures of 80° or above for preparing rice starch substrates will give the maximum rate of soybean enzyme hydrolysis.

The decrease in the rate of soybean amylase action on corn, wheat, and potato starches which have been heated above the optimum temperatures is an interesting phenomenon. However, after 24 hours' digestion the different starches prepared at these higher temperatures approach about the same limits of maltose formed as the same starches heated at their optimum temperatures. These results suggest that there is some effect on the starches when heated, other than the swelling and rupture of the granules. It is possible that heating causes agglutination of the particles in the gelatinized starch paste, so that a change in the degree of dispersion occurs. The individual starch molecules would then be less accessible to the attack of the soybean amylase. The result would be a retarding effect, but eventually the same degree of hydrolysis would be accomplished.

Summary

A potentiometric method for determining reducing sugars quantitatively which is applicable to the determination of maltose formed during enzymic hydrolysis of starches has been described.

The temperature at which the starch substrate is prepared has a definite effect on the rate of soybean amylase action.

The optimum temperature of preparing starches for soybean amylase action depends on the kind of starch to be used. The following temperatures of gelatinization were found to give the maximum rates of soybean amylase action on the different starches studied—70° for potato, 80° for tapioca, 90° for wheat and corn starches, and 80° or above for _____ starch.

A possible explanation of the retarding effect of heating above these optimum temperatures on soybean amylase action on corn, potato, and wheat starches is given.

Acknowledgment

The authors wish to thank Nellie M. Naylor and R. M. Hixon for their advice and suggestions during the course of this work.

Literature Cited

- Blish, M. J., and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* 10: 189-202.
- Day, E. D.
1908 Digestibility of starches of different sorts as affected by cooking. U. S. Dep. Agr. Off. Exp. Sta. Bull. 202.

- Ford, John S.
1904 Lintner's soluble starch and the estimation of "diastatic power." *J. Soc. Chem. Ind. (London)* **23**: 414-422.
- Furman, N. H., and Evans, O. M.
1929 Applications of ceric sulfate in volumetric analysis. V. Potentiometric study of the reaction between ferrocyanide and ceric ions. *J. Am. Chem. Soc.* **51**: 1128-1133.
- and Wilson, E. B., Jr.
1928 A simple continuous reading method of electrometric titration with bimetallic electrodes. *J. Am. Chem. Soc.* **50**: 277-283.
- Glock, G. E.
1936 An investigation of the rates of digestion of starches and glycogen and the bearing on the chemical constitution. I. Action of amylases on starches and glycogen. *Biochem. J.* **30**: 1386-1396.
- Gore, H. C., and Steele, H. K.
1935-6 Estimation of saccharifying power of malt diastase. *Ind. Eng. Chem. Anal. Ed.* **7-8**: 324-326.
- Hassid, W. Z.
1937 Determination of sugars in plants by oxidation with ferricyanide and ceric sulfate titration. *Ind. Eng. Chem. Anal. Ed.* **9**: 228-229.
- Hermano, A. J., and Rask, O. S.
1926 A consideration of certain reactions of starches with special reference to enzyme hydrolysis. *Cereal Chem.* **3**: 361-392.
- Nagao, Y.
1911 Vergleichende Studien über die Einwirkung von Pankreas diastase auf Hafer- und Weizen Starke. *Z. Exp. Path. Ther.* **9**: 227-237.
- Ohlsson, E., and Edfeldt, O.
1933 Amylasen in ruhenden und keimenden Samen. III. Hafer. *Z. physiol. Chem.* **221**: 174-179.
- O'Sullivan, J.
1904 A comparison of the products of the hydrolysis of potato starch with those obtained from cereal starches. *J. Chem. Soc.* **85**: 616-623.
- Sherman, H. C., Walker, Florence, and Caldwell, Mary L.
1919 Action of enzymes upon starches of different origin. *J. Am. Chem. Soc.* **41**: 1123-1129.
- Stone, W. C.
1896 The carbohydrates of wheat, maize, flour and bread and the action of enzymic ferments upon starches of different origin. *U. S. Dep. Agr. Off. Exp. Sta. Bull.* **34**.

SOME PHYSICAL FACTORS AFFECTING THE DISPERSION OF SOYBEAN PROTEINS IN WATER

R. H. NAGEL, H. C. BECKER, and R. T. MILNER

U. S. Regional Soybean Industrial Products Laboratory, Urbana, Illinois ¹

(Received for publication January 14, 1938)

It is the object of this paper to consider some of the factors which might influence the amount of nitrogenous constituents dispersed in water from fat-free soybean meal and to point out analytical precautions that should be taken when the measurement of this dispersion is attempted.

Considerable data have appeared in the literature (see Bailey, Capen, and LeClerc, 1935; Osborne and Campbell, 1898; and Staker and Gortner, 1931) giving values for the solubility of seed proteins. In most of this previous work the main objective has been to isolate protein fractions and but little consideration has been given to the physical factors which might affect the extraction. A few investigators have realized the significance of these conditions and have published important observations on them. Johns and Jones (1916) noted that there was no appreciable difference in the amount of protein extracted from peanut meal by sodium chloride solutions at 40 to 50° C. and at room temperature. On the other hand O'Hara and Saunders (1937) show that variations in temperature affect the amount of protein extracted from orange-seed meal by 4 N. sodium chloride solution. They also present data to show that within reasonable limits, time is not an important factor in the extraction of flaxseed proteins. Bishop (1929) observed that about 10% more protein was extracted from barley when it had been finely ground. Hofman-Bang (1930) also working with barley states that in order to obtain uniform results the meal should be ground in a ball mill until 95% passes through a 100-mesh sieve. Staker and Gortner (1931) also realized the importance of fine grinding and a standard particle size. They ground the meal of various seeds to pass through a 100-mesh sieve before extraction, but presented no data to justify this choice.

In the work reported in this paper the amount of nitrogenous material dispersed was measured by a Kjeldahl determination on the suspensoid. It has been generally assumed by investigators in this

¹ A cooperative organization participated in by the Bureau of Chemistry and Soils and Plant Industry of the United States Department of Agriculture, and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

field that by far the greater part of the nitrogen exists in the form of proteins. Winton (1935) presented a compilation of the work of three groups of investigators who reported the nitrogen distribution of soybeans. Their work indicates that approximately 5 to 10% of the nitrogen is non-protein.

In a preliminary survey samples of a number of varieties grown in widely separated localities were extracted, but it was soon discovered that variations in the analytical procedure could cause greater differences in the results than were observed between the varieties. Hence, it was considered necessary to investigate first the analytical method.

The soybeans used in this work were Illini variety grown during 1936 near St. Joseph, Illinois. They were cracked, flaked, and then extracted with petroleum ether (boiling range 35 to 60°) in a Soxhlet-type apparatus. The fat-free meal was air dried at room temperature, and ground. In order to avoid the possibility of heat denaturation, the temperature of the meal was kept below 40° C. at all times.

In grinding, it was noted that a burr-type mill would allow the relatively tough, thin hulls to pass through without reducing them to as small a size as desired. A burr grinder is also objectionable because of the danger of altering the proteins by frictional heat. In this work a porcelain ball mill with flint pebbles, or a Wiley mill, was used.

It was observed that the hulls are much harder to grind and that the particles retained on the coarser screens consisted largely of hull fragments. In order to test the solubility of the nitrogen compounds in the hulls, a sample of hulls was carefully separated by hand from oil-free flakes. An analysis of the hulls is presented in Table I and the amount of nitrogenous compounds extracted is shown in Table III. Although the hulls comprise about 6 to 8% of the total weight of the soybean (see Bailey, Capen, and LeClerc, 1935), they contain less than 1% of the total nitrogen, and as shown in Table III this small amount is not easily dispersed in water. These factors coupled with the tendency of the hulls to segregate make it imperative that thorough mixing and careful sampling be employed if concordant results are desired.

Before investigating the effect of each variable the tentative procedure given below was adopted. This procedure is similar in many respects to the method used by Staker and Gortner (1931) in their work on extracting protein from seeds. A weighed amount, usually 2.5 g., of the fat-free meal was shaken with distilled water at room temperature for 30 minutes on a mechanical shaker. The mixture was then centrifuged for 6 minutes at about 3,000 r.p.m., and the liquid was carefully decanted or filtered into a Kjeldahl flask and analyzed for nitrogen.

The residue was again shaken with another portion of water for an equal length of time, and the liquid was analyzed as before. This process was repeated as many times as was considered necessary (usually three).

Following this general procedure, an investigation was made of the effect of the size of the meal particles, the solvent-meal ratio, temperature, time, and separation of the meal residue from the suspensoid.

1. *Size of meal particle.* Five different sizes of meal were prepared from the same batch of flakes. Table I lists the moisture and nitrogen content of each of these five sizes and demonstrates that the grinding and sifting process to which they were subjected did not appreciably change the samples in these respects. The results of duplicate determinations are given in Tables I and III to indicate the precision obtained.

Size No. 1 meal consisted of the whole flakes, including hulls, and was the batch from which the other sizes were obtained by grinding. A screen analysis of the meal prepared in the Wiley mill is given in Table II. Sizes Nos. 2, 3, and 3A were prepared by means of a Wiley

TABLE I
ANALYSIS OF SAMPLES OF SOYBEAN MEAL PREPARED FOR EXTRACTION

Size number and description of particles	Moisture	Nitrogen	Total nitrogen in 2.5 g. sample
	%	%	Mg.
Hulls only	10.35	1.44	36.0
No. 1, flakes (including hulls)	9.46	8.19	204.0
	9.48	8.14	
No. 2, Wiley, through 2-mm. openings	9.53	8.05	200.3
	9.51	7.98	
No. 3, Wiley, through 1-mm. openings	9.39	8.21	204.5
	9.41	8.15	
No. 4, ball mill, through 100-mesh screen (.147-mm. openings)	9.42	8.21	204.3
	9.42	8.13	
No. 5, ball mill, through 200-mesh screen (.074-mm. openings)	9.38	8.17	203.8
	9.55	8.14	
No. 6, wet grind in ball mill, No. 4 meal used	9.42	8.21	204.3
	9.42	8.13	

mill using the 2, 1, and .5 mm. screens, respectively. Sizes Nos. 4 and 5 were obtained by grinding in a ball mill until all the material passed through 100- and 200-mesh screens, respectively. During the ball mill grinding the meal was sifted every few hours, the portion that had passed through the specified sieve being removed each time. It required about 24 hours to grind a sample until it would all pass through the 100-mesh screen and 48 hours to reduce to the 200-mesh size.

In addition to these five samples one run was made (No. 6 in Table III) by wet grinding in the ball mill. A 10-g. sample of size No. 4

meal plus 400 ml. of water was ground for $4\frac{1}{2}$ hours. The mixture was then centrifuged, filtered, and a 100 ml. aliquot, representing 2.5 g. of meal, taken. Table III shows the amount of nitrogen extracted from these various-sized meals.

TABLE II
SCREEN ANALYSIS OF 100-GRAM SAMPLES OF SOYBEAN MEAL

Size	Meal retained on mesh size—					Meal passed through mesh size—	Total recovered from sample
	20	35	60	80	100	100	
	<i>G.</i>	<i>G.</i>	<i>G.</i>	<i>G.</i>	<i>G.</i>	<i>G.</i>	<i>G.</i>
No. 2	0.6	34.5	38.7	6.7	2.2	17.1	99.8
No. 3	None	13.4	47.2	14.0	4.7	20.5	99.8
No. 3A	None	None	1.1	3.4	6.3	88.4	99.2

TABLE III
NITROGEN EXTRACTED BY 100 ML. OF WATER FROM 2.50-GRAM SAMPLES OF SOYBEAN MEAL OF VARIOUS-SIZED PARTICLES AS DESCRIBED IN TABLE I

Number of extraction	Nitrogen extracted from samples designated—						
	Hulls	1	2	3	4	5	6
	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>
First	8.2	130.0	139.5	146.5	165.3	160.8	189.1
		133.2	139.5	148.7	165.7	161.3	189.2
Second		27.6	23.4	20.9	17.8	17.1	—
		27.6	22.0	22.3	18.1	17.8	—
Third		5.6	4.2	4.2	3.8	2.2	—
		6.3	4.0	4.5	3.7	2.4	—
Total ¹		165.1	166.3	173.5	187.1	180.7	189.1
Total extracted, %		80.9	83.0	84.8	91.6	88.7	92.6

¹ Results with duplicate samples were averaged and then totaled.

From the data given in Table III it may be readily seen that the size of the meal particle has an appreciable effect upon the amount of protein dispersed. As would be expected, the solubility increases with a decrease in particle size except that the 200-mesh meal is slightly less soluble than the 100-mesh. This unexpected result can be accounted for on the basis that the long pounding in the ball mill, necessary to reduce the meal to 200-mesh, has caused a denaturation of the protein. Smith, Circle, and Brother (1938) have noted a similar effect upon the solubility in salt solutions.

On the basis of the greatest amount of nitrogen extracted with a minimum of labor, the method of wet grinding in a ball mill is the most efficient. Although this procedure apparently has not been employed

in analytical studies on the extraction of seed proteins, it appears to offer distinct advantages for this purpose. A very thorough grinding action is obtained and the liquid prevents local overheating and mechanical shock, both of which apparently cause denaturation.

Of the meal particle sizes studied, size No. 4 (100 mesh) was the most satisfactory. It not only yielded a large amount of protein in dispersion but it was also much easier to throw down in centrifuging, thus permitting a clean separation by decantation. The difference between size No. 4 and coarser meal is appreciable in this respect and is of itself adequate reason for adopting the finer size. In the dry grinding of soybean meal it is not practical to attain a limit of fineness more than about 100-mesh. This size meal was used in all subsequent work reported in this paper. Size No. 3A (.5 mm. Wiley) has been found to give practically the same results (see Smith, Circle, and Brother, 1938) as 100-mesh meal. This is to be expected from its screen analysis as shown in Table II.

A few preliminary experiments using 5 and 10% sodium chloride solutions showed that these conclusions hold equally well for salt solutions. It was also noted that pure water removes slightly more nitrogen than the salt solutions tried. Quantitative data on this subject will appear in another publication (Smith, Circle, and Brother, 1938) from this laboratory.

2. *Solvent meal ratio.* In consideration of the analytical procedure involved it was decided that a 2.5-g. sample of meal was best suited for the purpose. A survey of the literature showed wide variations in meal to water or salt solution ratios, with no data to justify the procedure in this respect.

Table IV indicates that there is nothing critical about the meal-water ratio, but some limits may be given. For quantities of water less than 50 ml. the resulting mixture is too viscous to centrifuge or filter readily, and the number of extractions required to remove the

TABLE IV
PERCENT NITROGEN EXTRACTED FROM SOYBEAN MEAL BY VARYING
AMOUNTS OF WATER

Number of extraction	Nitrogen extracted by water in amount of—				
	25 ml.	50 ml.	100 ml.	200 ml.	400 ml.
	%	%	%	%	%
First	59.4	73.6	81.0	80.8	82.7
Second	22.4	13.9	9.3	10.1	5.5
Third	5.8	4.0	1.9	1.1	.6
Fourth	1.6	.6	.2	—	—
Total	89.2	92.1	92.4	92.0	88.8

same percentage of protein is greater. On the other hand, large amounts of water did not remove appreciably more nitrogen and are difficult to handle in the nitrogen analysis. It is concluded that a ratio of water to meal of 100 to 2.5 by weight is satisfactory for this extraction. Should it be desirable to keep the total amount of liquid as low as possible, a greater number of extractions with small portions may be used. For example, four 25-ml. portions extracted as much protein as did three 400-ml. portions with a difference of 1,100 ml. in the water used. A comparison of the first extractions, however, shows that the 400-ml. portion removes considerably more than smaller amounts.

In making successive extractions such as described, there will always be some water remaining with the meal. This was from 7 to 10 ml. for 2.5 g. of meal. It is obvious that this water has dispersed its share of protein and that this fraction is carried over to appear in subsequent extractions. In no case investigated does this carry-over account for the total recovered from following extractions although it is responsible for a large share.

In order to determine the effect of moisture content, two samples of No. 4 meal were placed in desiccators. One desiccator contained anhydrous calcium chloride, the other a sulphuric acid water mixture adjusted to maintain a relative humidity of 75%. After three days one sample contained 2.93% and the other 15.61% moisture. The percentage nitrogen extracted from each of these samples was within 2% of that reported in Table III for meal containing 9.42% moisture. Hence, it is concluded that within this range the moisture content of the meal is not a factor influencing the extraction of nitrogenous compounds with water.

3. *Temperature.* Table V shows the effect of temperature variation on the amount of nitrogenous compounds dispersed.

TABLE V
PERCENT NITROGEN EXTRACTED AT DIFFERENT TEMPERATURES

Temperature	Nitrogen
° C.	%
1.5	71.4
14.0	76.3
28.0	80.0
35.0	81.6
45.0	83.1

It can be readily noted that temperature has a definite effect and that extreme variation in temperature will cause discrepancies in the results. In the range of 15 to 35° C. the variation is about 0.25% nitrogen per degree C. The room temperature in this series of experi-

ments averaged 27° C., and it is estimated that variations of not over 1% have been introduced as a result of this factor

4. *Time.* Table VI indicates the effect of time variations in the shaking period on the amount of nitrogen extracted.

TABLE VI
PERCENT NITROGEN EXTRACTED FROM SOYBEAN MEAL, WITH VARIATIONS IN THE
TIME OF SHAKING

Number of extraction	Nitrogen extracted when sample was shaken—							
	1 min.	7½ min.	15 min.	30 min.	60 min.	120 min.	180 min.	240 min.
	%	%	%	%	%	%	%	%
First	71.9	75.6	78.0	81.1	80.7	82.3	83.6	83.9
Second	18.1	14.9	—	8.8	—	8.1	8.2	—
Third	2.6	1.9	—	1.8	—	1.4	1.4	—
Total	92.6	92.4	—	91.7	—	91.8	93.2	—

The length of time spent in shaking, like the water-meal ratio, is not at all critical. The results presented indicate that 1 minute's shaking for each of three extractions will disperse as much protein as 3 hours for each extraction. The amount extracted on the first shaking is considerably greater, however, for the longer interval. It should be remembered that the time spent in shaking was not the entire period that the meal was in contact with water. It was found that about 10 minutes were required from the time shaking was stopped until the liquid had been decanted.

A consideration of the mechanics by which a colloidal sol such as this is formed leads to the conclusion that either the protein particle will float away readily from a broken cell or the cell walls must be ruptured by the softening and swelling action of the solvent. The alternative of a migration through unbroken cell walls is rejected as being improbable for a particle having the size of protein molecules. The rapidity with which the dispersion takes place from 100-mesh meal leads to the conclusion that there are but few cell walls which have escaped damage during the grinding process. This conclusion is not in accord with Davidson (1929) who claims that reduction to 200-mesh is necessary to assure that all the cell walls are broken. His criterion, however, was the tensile strength of an adhesive produced after admixture with other substances. It may be noted from Table III that the second and third extractions yield a greater return of soluble nitrogen compounds from the coarser meal sizes, probably because of the time required by the solvent to force open unbroken or only slightly damaged cells.

5. *Separation of meal residue from suspensoid.* After shaking the meal with water the problem of separating the liquid from the suspended meal must be solved. Even after centrifuging for 10 minutes at 3,000 r.p.m. one can not be assured that minute meal particles are not still suspended. After centrifuging, the liquid has a slightly turbid appearance but no particles are visible to the unaided eye. Filtering through No. 1 Whatman filter paper did not remove any additional nitrogen as compared with the liquid decanted from the settled meal. Hence, it was considered justifiable to separate this liquid by simple decantation especially in the case of the 100-mesh meal which settles out very readily upon centrifuging.

To indicate the particle size, the suspensoid after centrifuging for 10 minutes as above was filtered twice through No. 1 Whatman filter paper and then centrifuged again, this time at 48,000 r.p.m. for 30 minutes in a supercentrifuge. A clear, slimy precipitate was thrown out removing 6.86% of the nitrogen from the liquid. The liquid was considerably clearer after this fraction had been removed. A more detailed study of this subject is contemplated and an attempt will then be made to arrive at a particle size distribution.

Summary

A study has been made of five physical factors influencing the amount of nitrogenous material extracted from soybean meal by water. The data presented may be used as a guide in establishing a procedure for the extraction by water of the proteins from soybean meal.

The ratio of water to meal, the temperature, and the time of extraction were found to exert small effects and the magnitude of these effects has been determined.

The size of meal particles is a very important factor. Meal passing through 100-mesh screen was found to be satisfactory for the purpose of extracting nitrogenous compounds. Wet grinding in a ball mill is suggested as an efficient method of dispersing soybean proteins.

Literature Cited

- Bailey, L. H., Capen, R. G., and LeClerc, J. A.
1935 The composition and characteristics of soybeans, soybean flour, and soybean bread. *Cereal Chem.* 12: 441-472.
- Bishop, L. R.
1929 Second report on barley proteins. Composition and quantitative estimation of the barley proteins. II. *J. Inst. Brewing* 35: 316-322.
- Davidson, G.
1929 U. S. Patent No. 1,724,695.
- Hofman-Bang, G.
1930 The influence of the method of grinding on the estimation of barley proteins. *J. Inst. Brewing* 36: 381-388.
- Johns, C. O., and Jones, D. Breese
1916 The proteins of the peanut, *Arachis hypogaea* L. I. The globulins arachin and conarachin. *J. Biol. Chem.* 28: 77-87.

- O'Hara, L. P., and Saunders, Felix
1937 Studies in proteins. VI. The solubility of the nitrogenous constituents of certain seeds in sodium chloride solutions. *J. Am. Chem. Soc.* **59**: 352-354.
- Osborne, Thomas B., and Campbell, George F.
1898 Proteids of the soybean. *J. Am. Chem. Soc.* **20**: 419-428.
- Smith, A. K., Circle, S. J., and Brothier, G. H.
1938 Peptization of soybean proteins. I. The effect of neutral salts on quantity of nitrogenous constituents extracted. *J. Am. Chem. Soc.* **60**: 1316-1320.
- Staker, Ernest V., and Gortner, Ross Aiken
1931 Physico-chemical studies on proteins. V. A comparative study of the peptization of the protein complex in various seeds and grains. *J. Phys. Chem.* **35**: 1565-1605.
- Winton, A. L., and Winton, K. B.
1935 Structure and Composition of Foods. Vol. I, p. 518. John Wiley and Sons.

A PRACTICAL AND SIMPLE METHOD OF RECORDING THE FORM AND POROSITY OF BAKED PRODUCTS

CH. MATĚJOVSKÝ

Milling Research Station, Prague, Czechoslovakia

(Received for publication January 16, 1937)

In carrying out practical baking tests it is useful to keep not only a numerical record of the measurements and assigned scores, but also a pictorial record of the form and porosity of the baked product. Heretofore these characteristics have been recorded by preserving and storing the product wholly or in part, by photographing the cut surface, or by spreading an ink over the surface of a slice and stamping an impression on absorbent paper. The impressions obtained by the latter method, however, are lacking in distinctness because of the distortion of the individual pores by the strong pressure exerted during the stamping operation. Moreover, the outlines of the individual pores are not clearly defined, and because of the uneven surface of the slice, many points in the impression are left blank.

If the primary object of these pictorial methods is to obtain a record of the size and form of the individual pores, the above-mentioned photographic method presents many difficulties, because the poor definition of the pores in the resulting photographs does not permit a practical judging of the baked product or an accurate comparison with Mohs' (1924) ¹ scale of porosity. It is also possible to ascertain the corresponding numbers on the Mohs scale either by direct comparison with the cut surface or preferably with the stamped impression, but the results have not been found satisfactory.

¹ Mohs, Karl. The size of the pores in baked bread. *Cereal Chem.* **1**: 149-151 (1924).

An improved method which the writer has introduced into the practice of the Milling Research Station is based upon making a contact print of a thin slice of the baked product directly upon a contrast photographic paper. A slice is cut from the product at least 12 hours

Brno II

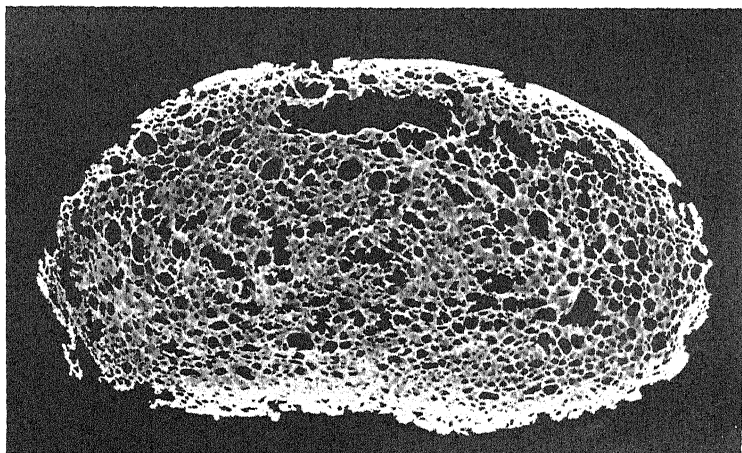


Figure 1

Dregerova 3D

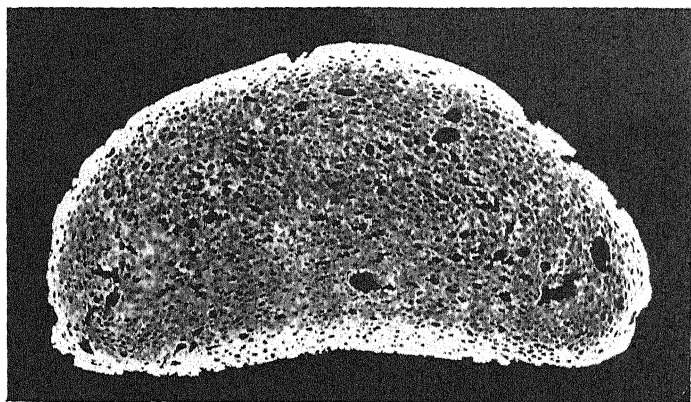


Figure 2

after baking, the smooth cut being made by means of a sharp knife or, preferably, a hand-driven slicing machine, of the type used in restaurants for slicing bread, bacon, etc. The machine is equipped with a cutting disc driven by a gear and a hand wheel. The mounting table can be moved in a vertical direction, and the thickness of the

slice can be regulated at will. The slices are cut to a thickness of 0.50 to 0.75 mm., and the crumb structure should be well preserved. Several slices are taken in this manner and the thinnest one showing the best preserved crumb structure is selected. As the slice is prone to

Perbete

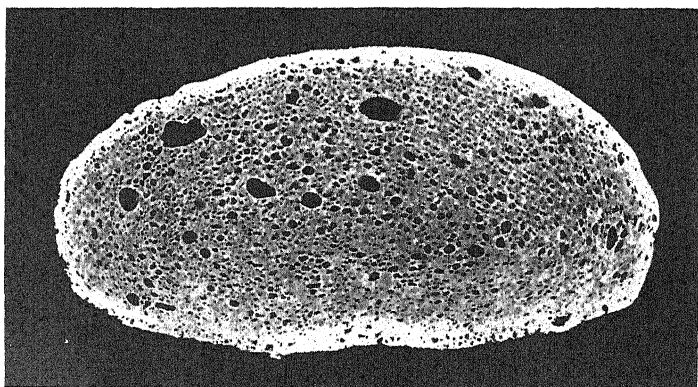


Figure 3

Stocken Slezsko

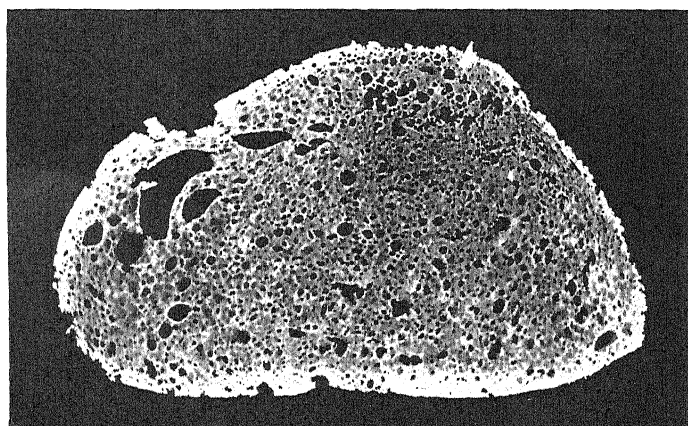


Figure 4

drying and curling, it is immediately placed between two glass plates, the lower of which is covered with a filter paper soaked in a 75% solution of glycerine in water. An excess of the solution must be avoided to prevent the filling of the minute pores of the slice. This method of treatment insures an absolutely flat slice.

The slice soaked in diluted glycerine is now taken to a dark room and transferred to a photographic paper of the proper size, which is placed upon a perfectly flat surface, such as a glass plate. Each slice may be provided with a celluloid label bearing the name and reference number of the sample in India ink. The photographic paper bearing the slice is now exposed to a suitable source of illumination, preferably a frosted lamp, placed 25 to 30 cm. from the paper, and given the proper exposure. The paper is then removed and developed and fixed in the usual manner. The photographic paper best suited for this purpose is a contrast developing paper with glossy or semi-glossy surface.

The procedure as described is very simple, rapid and inexpensive. The prints furnished by this method present a great advantage over those obtained by stamping the impression of the inked slice, as the pores of the baked product are clearly defined and free from any distortion due to strong pressure. The prints (see Figures 1 to 4) show the shape of the loaf as well as the thickness of the crust.

By this method for rapid recording of the structure of baked products in laboratory practice, a number of prints may be collected and used as a basis of comparison in commercial and forensic analysis. A scale of porosity resembling that of Mohs could be set up, using numbers from 1 to 10 to denote increasing pore size. Such an improved scale would permit more accurate and easier comparisons, and the difficulties encountered in attempting to relate imperfect impressions to the individual types of porosity shown in the Mohs scale would be eliminated.

SOME PHOTOMICROGRAPHIC STUDIES OF DOUGH AND BREAD STRUCTURE

S. WALTER BUTTERWORTH and W. J. COLBECK

College of Technology, Leeds, England

(Received for publication August 7, 1937)

Earlier work on the subject was carried out by Scheffer and published in 1916. Scheffer stated he was unable to discover previous work in this field. He observed that the paraffin and gelatine embedding methods did not give satisfactory results for the cutting of thin sections of bread, and recommended cutting the semi-dried bread-stuff by hand microtome using an oblique cutting action. If the sample had hardened or dried out too far, it was softened slightly by keeping in a moist atmosphere containing formaldehyde to prevent mould growth. The section was examined in a mixture of glycerine and water and much information was gained without staining the specimen. The use of polarized light was also recommended. A mixture of methyl green and cresol violet was suggested as particularly suited to the examination of wholemeal bread because the different parts of the bran of the grain are differentially stained by these reagents. Scheffer illustrated his paper with photomicrographs.

The subject was taken further by J. R. Katz and E. Van Teutem who published the results of their investigations in 1917. Bread slices were cut after embedding in gelatine and no photomicrographs were given, the paper being illustrated by diagrams by E. Van Teutem. A gluten structure with starch and yeast embedded was observed. The starch cells adjacent to the air cell surfaces were elongated or "bean" shaped, while those further within the breadstuff appeared to retain their original shape. A comparison with the structure of a cut-steel surface was made, where all the particles appear to be oriented by the flow of the metal before setting. It was further stated that in the bread from correctly fermented doughs, the starch cells present a regular formation, the larger cells being interspersed with smaller cells, while in bread from overripe doughs the degree of dispersion is greater, the starches are more widely separated and all formation is lost. In underripe dough the cells are closer, overlapping, and again show no regular formation. In underfermented and overfermented loaves it is stated that a lower proportion of gluten is to be seen.

The work of Verschoffelt referred to by Katz (1917) showed the development of air layers around the starch cells in old bread and this phenomenon was illustrated diagrammatically by E. Van Teutem. The irregular shape of starch cells in old bread was compared with the rounded cell forms existing in fresh bread.

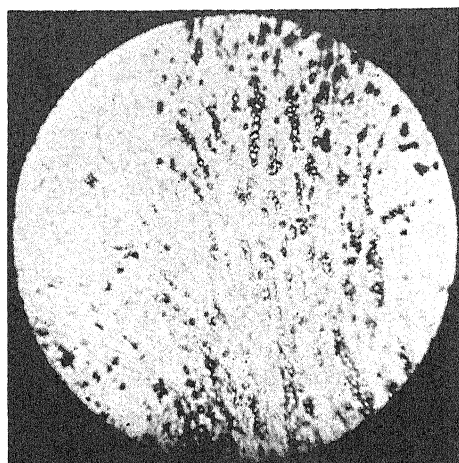


Figure 1. Gluten film (low power). $\times 100$.

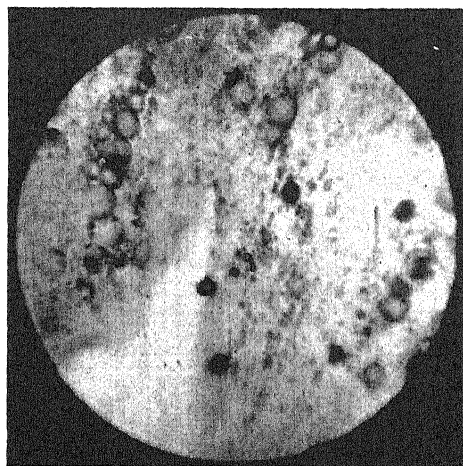


Figure 2. Gluten film (high power). $\times 400$.

The present work was undertaken with the object of observing dough and bread structure, developing different micro-methods of investigation and obtaining photomicrographic records of the observations made. Work on gluten films was first carried out, and Figures 1 and 2 show the gluten films obtained by continued stretching of

hydrated gluten. In Figure 1 (low power), the starch cells were stained with iodine and the film allowed to dry (no cover slip was used). The uniform order of the starch cells in Figure 1 shows the strain to which

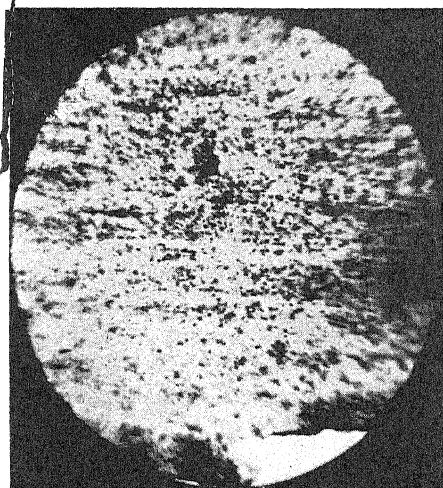


Figure 3. Gluten film (low power). $\times 60$.

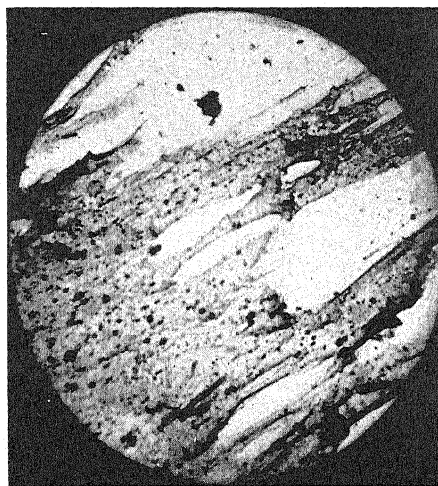


Figure 4. Gluten film (low power). $\times 60$.

the gluten was subjected during stretching. Figure 2 (under higher power) shows the disposition of the starch cells more clearly. It is interesting to note that in Figures 1 and 2 there is little evidence of

the presence of the gluten film. Only in Figure 2, where an edge of the film is shown, is the gluten visible. Figures 1 and 2 are not stained

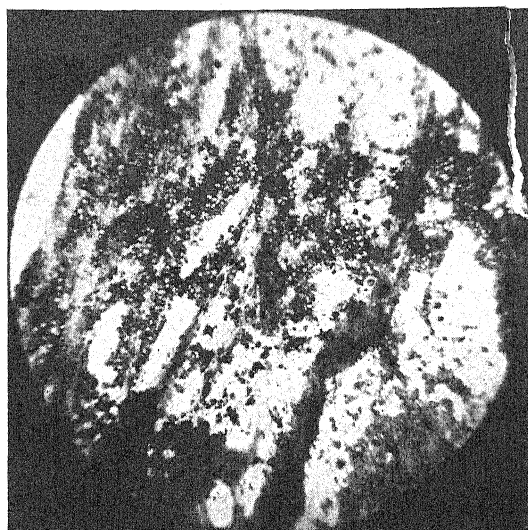


Figure 5. Sponge strand (low power). $\times 100$.

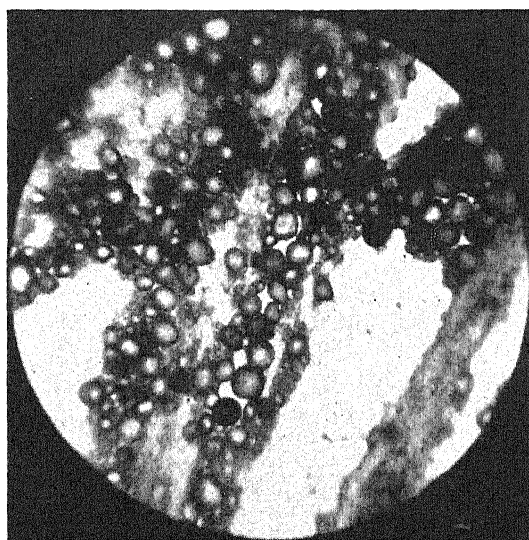


Figure 6. Sponge strand (high power). $\times 400$.

with eosin but even when such a stain is used the presence of a thin gluten film is not easily detected. Figure 3 exhibits the same phenomenon and Figures 1, 2, 3 and 4 support the view that gluten is

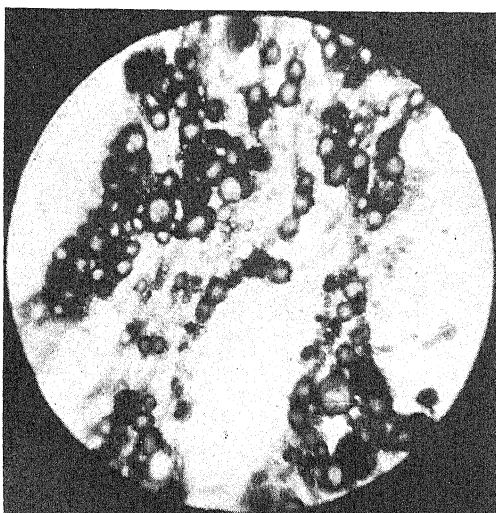


Figure 7a

Correctly fermented dough film (high power). $\times 400$.

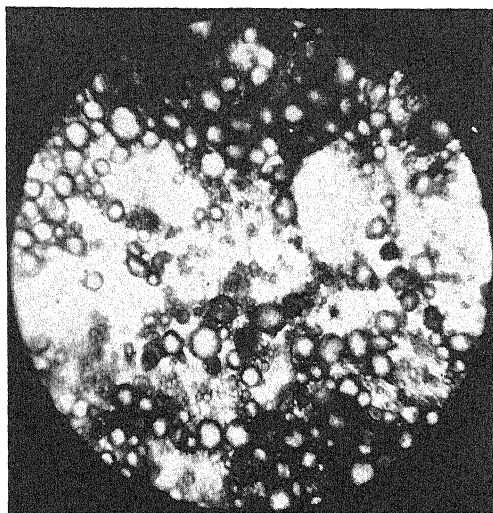


Figure 7

devoid of organized structure so far as can be ascertained microscopically. Figures 3 and 4 are further low-power photomicrographs

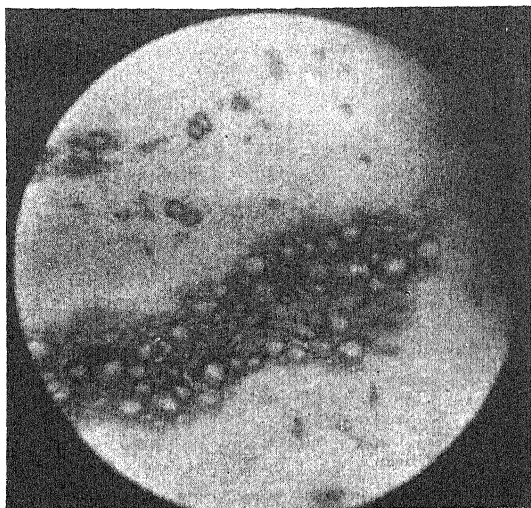


Figure 8. Overfermented dough film (high power). $\times 400$.

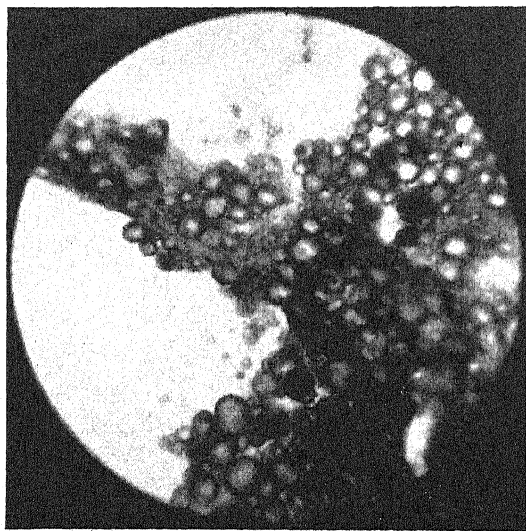


Figure 9. Underfermented dough film (high power). $\times 400$.

showing the pull to which the gluten films were subjected, Figure 4 showing the broken edge of such a film.

The stretching method was applied to sponges and also to doughs.

July, 1938 S. W.

as taken just before the dropping point and a thin strand of dough was placed on a microslip which gave the results shown in selected arand 6. This was stained with eosin and iodine but received no further treatment; the gluten structure is clearly seen supporting starch cells, some of which are partially embedded in the gluten mesh along with yeast cells. It will be observed also that some starch cells have taken the iodine stain and show up black of the the unruptured cells are unstained. Figure 5 shows the low-power field and Figure 6 a section of the same field under higher power.

Figures 7, 7A, 8, 9 and 10 were taken from dough films. Figures 7 and 7A (high power) show correctly fermented dough. A section was made when the dough was ready for the divider, a short time allow-

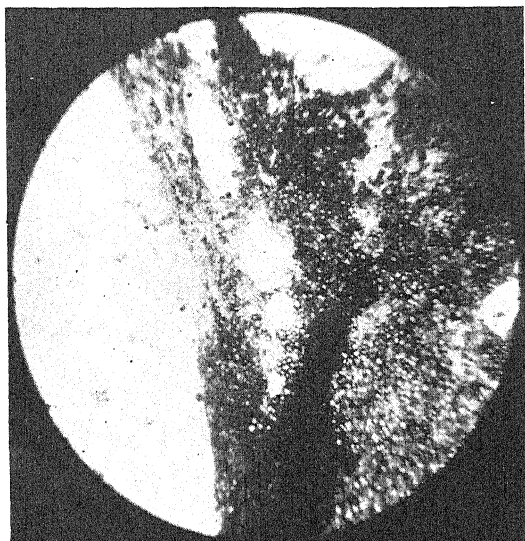


Figure 10. Underfermented dough film (low power). $\times 100$.

ance (15 minutes) being made for the preparation and staining so that the dough was correctly fermented when the photograph was taken. Figure 8 shows the structure of an overfermented dough and Figures 9 and 10 that of an underfermented dough under high and low power, respectively.

The photomicrographs of dough are typical of many fields examined. No evidence was discovered in support of the view that varying periods of fermentation give rise to characteristic changes in the arrangement of starch cells. Regular and irregular starch formations were observed in underfermented, correctly fermented and overfermented doughs and such formations would therefore appear to be of

little value in determining the degree of dough fermentation (see Figures 7, 7A, 8, 9 and 10).

It would appear that under the normal conditions existing in a fermenting sponge or dough, a much smaller proportion of starch is truly "embedded" in the gluten than had been previously considered to be the case.

It is probable that immediately after mixing or processing (dividing, moulding, etc.) much starch is embedded in gluten, but as fermentation proceeds the mechanical effect of the gas produced upon the gluten structure brings about a "squeezing out" of the starch cells from the gluten so that the cells adhere to the surface of the gluten mesh in

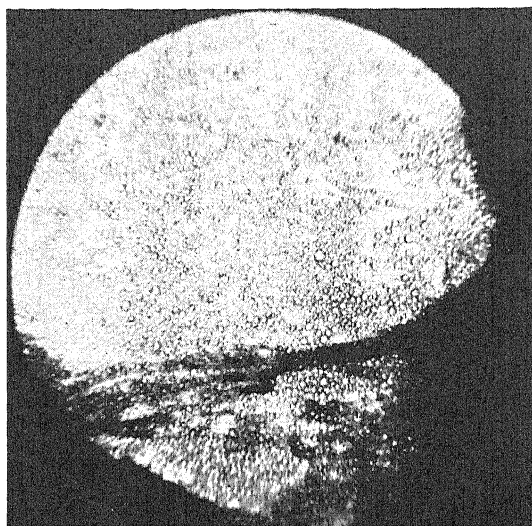


Figure 11. Disturbed dough film (low power). $\times 100$.

increasing proportions, the quantity embedded in the gluten decreasing accordingly.

During the course of the work on sponges and doughs it was found that ordinary methods of staining and preparation were unsuitable because such methods interfered greatly with the true structure of the material examined. Further it was observed that when a sponge or dough film was teased out with a mounting needle in the presence of a liquid (stain, water or glycerine), starch cells in considerable numbers were readily detached from the gluten structure and dispersed in the liquid. Such cells then concentrated against the first gluten strand, forming a barrier to the flow of the liquid. If such a slide was allowed to dry various starch formations, regular and irregular,

could be obtained and for some time such formations were difficult to account for.

Figure 11 (low power) shows starch cells floated off from their normal position by stain concentrated against a gluten strand. The

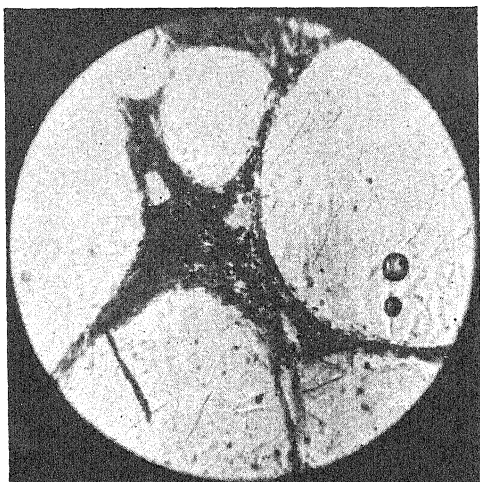


Figure 12. Bread section (low power). $\times 60$.

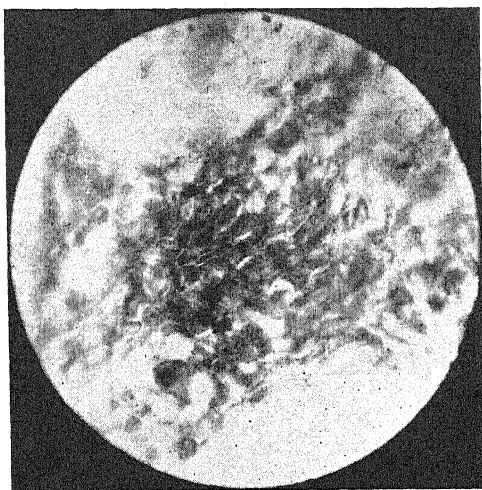


Figure 13. Bread section (high power). $\times 240$.

photograph was taken while the stain was still liquid. Although much of the starch was lost in the manner described many starch cells can be seen adhering to the gluten strand in their original positions, the

proportion dispersed depending upon the amount of manipulation or "teazing out" received by the dough.

It was found to be essential in the work described to observe the material with the minimum of preparation and staining, otherwise distorted results were obtained; the simplest technique produced the most accurate results.

After the experience gained from gluten, sponge and dough films, work on bread was undertaken. Figures 12, 13 and 14 show sections of bread cut by means of the freezing microtome after the small bread cubes had been hardened in formalin, and embedded in increasing gelatine concentrations. Figure 12 (low power) shows the starch cells stained with iodine within the texture of the bread, while Figures 13 and 14 show sections of the field under higher power. The gluten

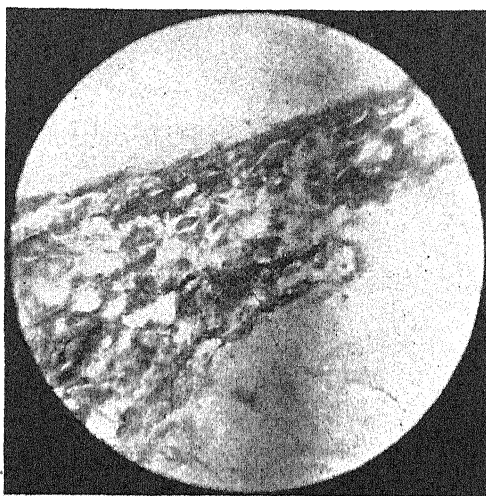


Figure 14. Bread section (high power). $\times 240$.

structure is shown up by the eosin stain, and the somewhat deformed shapes of the starch cells and the "bean shape" referred to by K¹ are also visible.

It was found difficult to cut sections sufficiently thin for ple micrographic purposes and it was also thought that the gelatine technique could not fail to give distorted results due to the prolonged soaking period and the formalin dehydration. Teazing out the breadstuff was therefore tried because under high power the breadstuff structure is observed and not the bread texture. It was thought that the manipulation of the breadstuff with the mounting needle might destroy the general texture but that the micro-structure of the breadstuff would remain undisturbed. The teazing out or disintegration of

the bread was only carried out to a degree which made microscopic observation under high power possible. Figure 15 shows the result of such teasing. Swollen starch cells and gluten masses are present but there is no sign of organized structure and the technique was abandoned because further work showed that even slight manipulation caused a disproportionate degree of disturbance, the phenomena observed being very similar to those previously referred to and illustrated in Figure 11. If a small amount of water or glycerine was used the disturbing effect of manipulation was intensified. A comparison of Figure 15 with the photomicrographs of sponge and dough structure

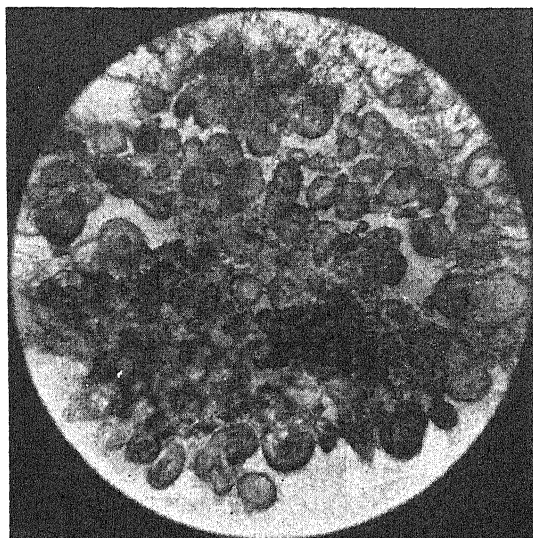


Figure 15. Bread section after "teasing." $\times 400$.

(Figures 5, 6, etc.) illustrates the increase in size of the starch cells due to baking, the high-power magnifications being identical.

Sections of one-day-old bread were then cut with a hand microtome, the bread slice being held in pith for support, these sections were stained with eosin and iodine and were examined without further treatment. The result obtained is shown in Figure 16. Only a small section of the field is in focus but that section shows the gluten strand formation in the bread and also the presence of overlapping starch cells. The resemblance of this small section to the stretched gluten film shown in Figure 3 may be noted.

Figure 17 shows another hand-cut section stained with eosin and iodine in dilute aqueous solution. The swollen and ruptured cells with the dark-stained gluten may be observed. The difference between Figures 12, 13 and 14 and Figure 17 is marked. The latter

photomicrograph shows no sign of elongated starch cells; it may be that this peculiar "bean" shape is due to one or another of the processes employed in the gelatine embedding.

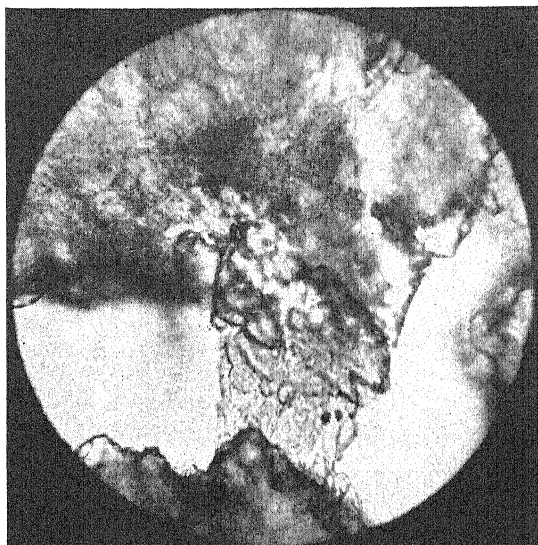


Figure 16. Bread section (low power). $\times 100$.

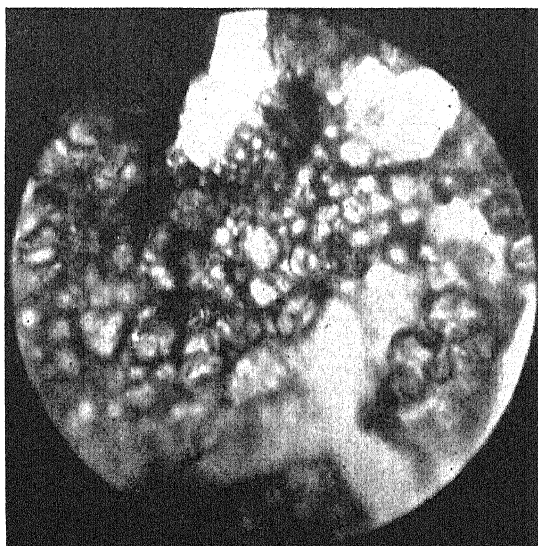


Figure 17. Bread section (high power). $\times 400$.

A number of experiments were carried out using various methods of preparing thin microscopic sections of bread. Figure 18 shows a

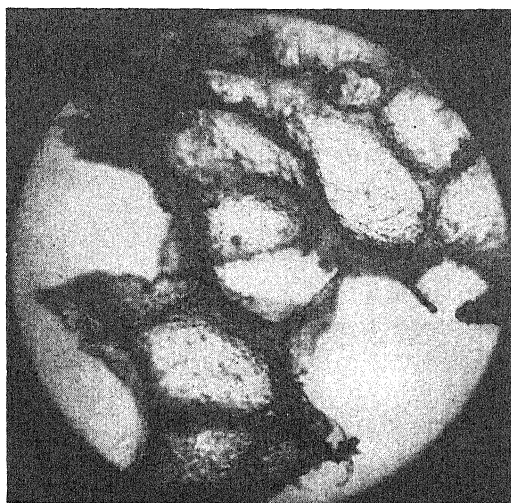


Figure 18. Bread section. Untreated. (Low power). $\times 100$.

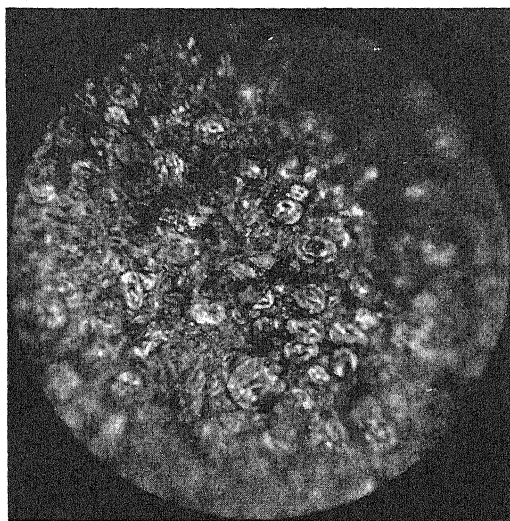


Figure 19. Bread section (high power). $\times 400$.

section of bread cut with a sharp knife. The thin slice of bread was cut from the loaf when approximately 12 hours old, the thinnest portion of this slice was selected, placed on a microslip and photo-

graphed without receiving any further treatment. The bread texture or vesiculation of the crumb is clearly seen.

Figure 19 shows bread structure as obtained by carefully selecting a section direct from the loaf and staining with iodine and eosin. The ruptured and unruptured starch can be seen; also overlapping starch cells and small yeast cells are visible.

Photomicrographic Procedure

A quarter plate camera was erected vertically above the microscope. Illumination was provided by a 60-watt gas-filled lamp 15 cm. distant from the microscope mirror. A substage condenser and mechanical stage were fitted. The plates used had a speed of 700 H and D, exposures ranging from 1 second to 6 seconds. The best results were obtained with 3 seconds exposure on the lower powers, and 5 seconds on the higher powers. For the low-power work 1 inch and $\frac{2}{3}$ inch objectives with either No. 2 or No. 4 oculars were used while a $\frac{1}{6}$ inch objective with similar oculars was found most suitable for high-power work.

Summary

Earlier work by Scheffer and Katz is reviewed.

Work on gluten films is described.

A study of sponge and dough films suggests that, as fermentation proceeds, the mechanical effect upon the gluten of the gas produced squeezes out the starch cells so that they adhere to the gluten surfaces in increasing quantities.

The material is best observed with the minimum of preparation, otherwise distorted results may be obtained.

No apparent differences are observed in the structure of dough at varying stages of fermentation.

Experimental work on the preparation of dough and bread sections is discussed.

Acknowledgment

The writers are greatly indebted to F. K. Birkett for the preparation of the gelatine-embedded bread sections.

Literature Cited

Katz, J. R.

1917 Onderzoekingen naar het oudbakken worden van brood en de middelen om dit te voorkomen. (Published by Dutch Government.)

Scheffer, W.

1916 Mikroskopische Dunnschnitte durch Gebacke. Z. ges. Getreidew. 8: 6-10.

A STUDY OF VARIOUS CHARACTERISTICS OF MILL-STREAM FLOURS AND THEIR RELATION TO LOAF VOLUME

R. H. HARRIS¹ and STEWART N. WHITE²

(Received for publication January 24, 1938)

Introduction

The importance of the various quality characteristics of mill stream flours needs no emphasizing to the mill chemist. These flours are the materials, as it were, from which the finished flour is constructed, and, as the quality of an edifice is very largely determined by the characteristics of its material, so the baking value of a flour is conditioned by the attributes of its constituent mill streams. These mill streams originate primarily from different portions of the wheat kernel: the flours of better color and low ash come from the more central portion of the endosperm, but with the gradual inclusion of material from the outer or branny layer, the color decreases and the ash content rises markedly. Even the best flours, of course, contain a small proportion of lower quality particles, such as bran, but these are present in relatively minute quantities. As the tail of the milling system is approached these impurities increase rapidly with a consequent influence upon flour quality. The situation in this instance is quite different from that encountered when dealing with flours milled from individual samples of wheat. The latter case is the one which cereal chemists are usually called upon to deal with, and quality variations may here be attributed to genetical or environmental differences in the wheat.

Bailey (1925) listed several analyses of roller mill streams published by Richardson (1884), Jacobs (approximately 1915), Swanson, Willard, and Fitz (1915), and Weaver (1921). The low ash content of the purified middlings was noted by these workers, who remarked also upon the tendency of this constituent to increase as the end of the milling system was approached. The protein percentages tended to increase in progressing through the milling system, while the percentage of dry crude gluten paralleled the nitrogen content rather closely. Loaves of high quality were baked from the break flours despite the lower gluten quality assigned these flours. Swanson and his co-workers found the highest color and texture in the first to sixth

¹ Cereal Technologist, North Dakota Agricultural Experiment Station, Fargo, North Dakota.

² Chief Chemist, North Dakota State Mill and Elevator, Grand Forks, North Dakota.

middlings flours respectively, and Weaver obtained the best bread from the streams lowest in ash. Gluten content was found to increase with ash in the middlings flours. This relationship did not exist in the break flours.

Pascoe, Gortner, and Sherwood (1930) examined a series of mill-stream flours from the Minnesota State Mill and found a negligible correlation to exist between flour protein and loaf volume as obtained by the Standard Basic Procedure advocated by Blish (1928). A relatively high correlation was obtained between these variables in a series of samples milled from different lots of wheat of the same grade. Marked variability was evident in the saccharogenic activities of the various mill-stream flours. The germ content was especially indicative of this activity, as pointed out by these researchers. The third break was lowest and the sizing flours highest in this respect. There was little relationship noted between the peptizable protein fractions and loaf volume.

Harris (1930) published the results of a study made on mill-stream flours produced from hard red spring wheat. This investigation dealt principally with the relations between flour protein, peptization, and baking strength. While total flour protein and loaf volume were highly related when the 12 best flours were considered, this relationship decreased to a point of little practical importance when the lower quality streams were included in the study. The use of a flour "improver" (3% malt and 0.5% Arkady) did not appear to be justified by the results obtained when compared with corresponding values yielded by the standard basic formula. A consideration of quantity of protein peptized in addition to a knowledge of total protein yielded slightly more information with respect to loaf volume for the entire set of twenty flours.

Rich (1934) thought that the presence of germ particles induced low grade quality in Canadian hard red spring wheat mill-stream flours, and attributed the beneficial effects of bleaching to oxidation of some germ constituent.

Later, Harris (1937, 1938) indicated the probable importance of the initial fraction precipitated from gluten dispersions in 10% sodium salicylate solutions by concentrated MgSO_4 solution as an index of the baking quality of North Dakota durum and hard red spring wheat flours. A separation of flours produced on an experimental mill to conform approximately to commercial mill streams was also studied, and the results obtained appeared to show a similar relationship in this instance also. The evidence obtained in the latter instance, due to the small number of samples involved as well as to the method of separating the various flours, was not considered sufficiently conclusive

by the author. Accordingly an investigation was undertaken to ascertain more definitely the relationships existing between the different factors of baking quality and loaf volume in a series of mill-stream flours produced in a modern commercial flour mill from a blend of sound bread wheat.

Experimental

MATERIAL AND METHODS

The mill streams described originated from various portions of the wheat kernels comprising a wheat mix of 1937-crop hard red spring wheat, the entire set or various combinations going to make up a finished product. This mix was cleaned, washed, and tempered in the normal procedure used in most mills and gave results as shown in the various reductions.

Two samples were taken direct from each stream; one in a small, air-tight container to secure analysis as actually milled; the other a two-pound bag sample for baking and further chemical examination. The total sampling time was approximately 30 minutes, which insured that no significant change in milling conditions occurred during the sampling period.

Moisture determinations on these samples were made by the air-oven method described on page 29 of the A. A. C. C. Cereal Laboratory Methods (1935).

Ash determinations were made by a modified direct burning method. The usual 5 g. of flour were used and a standard electric muffle furnace was used for burning. To shorten the burning time, a small amount of oxygen was kept flowing into the ignition chamber of the furnace through a very fine jet (approximately the size of a small hypodermic needle). To insure a constant flow of oxygen, about 10 pounds of pressure per square inch were maintained on the oxygen line. The jet is constructed in such a way that it disperses the oxygen into the oven without blowing any flour from the dishes. Flat silica dishes, of 15 c.c. capacity, 47 mm. diameter, and 13 mm. depth, were used for ashing the flour. The flour was ignited at 800° F. and the volatile matter burnt off before closing the oven. The oven was then closed and raised to 1100° F., and burning continued for two hours. This method has been found to approximate closely results obtained by the usual 5 to 6 hour method.

Protein determinations ($N \times 5.7$) were made on the samples according to the standard method outlined in Cereal Laboratory Methods.

The diastatic activity of the flours was determined using the Blish-Sandstedt (1933) method. The results are reported as milli-

grams of maltose produced in 1 hour from 10 g. of flour at 30° C. The resistivity of the starch substrate was also found, using a modification of the procedure developed by Malloch (1929), and contained in Cereal Laboratory Methods, pages 58-59. This modification was used by Shellenberger and Bailey (1936) in an investigation of the malting and biochemical properties of different varieties of barley, and differs chiefly from the original Malloch method in that three washings are made with distilled water without buffer solution, and in the digestion, which is carried out at the same temperature as the determination of diastatic activity (30° C.) rather than 27° C. Five grams of flour are used instead of 10 g. as proposed by Malloch. The glutens were washed from these flours by the procedure followed by the senior author in previous fractionation work (Harris, *loc. cit.*), and dispersed in 10% sodium salicylate solution. The gluten protein thrown down by one addition of concentrated MgSO_4 solution was determined using the technique and concentrations developed in this previous work.

The flours were baked by two procedures. One was essentially the Standard Basic Procedure mentioned by Blish (1928) as a reference method. The method used in this instance, however, contained 5% sucrose instead of 3%, as called for in the original basic procedure. The second method employed was the malt-phosphate-bromate formula in which 0.3% Lintner malt, 0.1% ammonium dihydrogen phosphate and 0.001% potassium bromate were included in addition to the ingredients used in the basic method. Three-hour fermentation periods were used throughout, and a two-minute mix in the Hobart-Swanson. The second method was used to bring out the full potentialities of the flours, and it was thought it would be especially valuable when baking the high gluten break flours. Harris noted a decided increase in the loaf volume of break flours when baking a series of hard red spring wheat mill-stream flours with an "improver" formula consisting of 3% malt and 0.5% Arkady. A marked increase in volume with the malt-phosphate-bromate formula was also remarked by Harris and Sanderson (1938) when working with hard red spring wheats grown in North Dakota in 1937.

Discussion

In Table I are shown the flour description, moisture, ash, protein, gluten content, and diastatic activity of the 27 samples included in this study. The moisture is highest in the initial break flours, tending to decrease as the tail of the milling system is approached. The duster flours contain material adhering to the bran which is relatively high in moisture and consequently raises the moisture content of these

flours above the average level for flours produced near the tail of the mill. The first four middlings flours are the lowest in ash, while the fifth break, low grade, and duster flours are very high in this constituent. A rather regular increase in ash is evident in progressing through the reduction system. This increase in ash is roughly paralleled by an increase in the protein and gluten content, which can doubtless be explained by the contamination of the lower grade of flours with material adjacent to the bran layer, which contains a

TABLE I
COMPOSITION OF FLOUR MILL STREAMS USED IN THE INVESTIGATION
(13.5% moisture basis)

Serial number	Mill stream	Moisture	Ash	Protein (N $\times$ 5.7)	Wet crude gluten	Protein (N $\times$ 5.7) in gluten	Gluten protein in flour	Diastatic activity
		%	%	%	%	%	%	Mg.
37-11- 4	1st break	16.2	0.50	13.3	47.8	26.1	12.4	188.9
5	2nd break	16.1	.44	14.8	51.5	27.0	13.9	137.3
6	3rd break	15.3	.55	16.6	63.0	25.5	16.1	135.8
7	4th break	15.2	.75	17.8	68.2	24.8	16.9	145.9
8	5th break	14.4	1.16	18.9	65.2	25.3	16.5	220.9
9	3rd break, No. 1	13.9	.43	12.3	43.7	25.4	11.1	214.4
10	3rd break, No. 2	14.2	.39	13.2	47.7	25.4	12.1	198.2
11	1st sizing	14.8	.63	12.1	41.3	25.5	10.5	340.1
12	2nd sizing	14.7	.40	11.8	41.5	27.2	11.3	418.4
13	3rd sizing	13.0	.47	11.8	40.5	26.3	10.6	237.4
14	1st middlings	14.3	.35	12.0	40.3	26.7	10.8	247.1
15	2nd middlings	14.3	.34	11.9	40.4	26.8	10.8	288.1
16	3rd middlings	13.6	.35	12.3	40.6	26.4	10.7	255.1
17	4th middlings	12.6	.38	12.8	42.6	26.9	11.4	257.7
18	5th middlings	12.8	.49	13.4	46.8	25.4	11.9	256.6
19	6th middlings	13.7	.54	14.0	49.7	25.4	12.6	220.7
20	7th middlings	13.3	.60	14.3	48.6	25.1	12.2	216.9
21	8th middlings	12.5	.67	14.1	48.3	25.9	12.5	244.8
22	1st tailings	13.7	.78	13.8	46.9	26.0	12.2	269.2
23	2nd tailings	13.6	1.08	15.5	54.2	24.9	13.5	235.8
24	3rd tailings	13.1	.74	12.9	48.3	26.2	12.6	261.6
25	1st low grade	12.4	.93	14.6	47.9	25.6	12.3	264.8
26	2nd low grade	11.6	.98	15.1	49.7	25.1	12.5	282.5
27	3rd low grade	12.1	1.15	15.5	49.0	25.1	12.3	280.5
28	Dust reel	13.3	.57	12.1	38.6	26.7	10.3	390.7
29	B & S duster top	14.3	1.47	16.8	56.2	25.3	14.2	329.7
30	B & S duster bottom	14.1	1.49	17.0	59.1	25.6	15.1	267.4

higher proportion of mineral and nitrogenous constituents. The saccharogenic activity is high in the sizing flours as noted by Pascoe, Gortner and Sherwood (1930) and was explained by them as due to the presence of germ material in these flours. The dust reel flour is also extremely high in diastatic activity and low in ash.

In Table II the fractionation and baking data are presented, with the results of the determination of the starch substrate resistivity to

enzymic attack. There does not appear to be any great variability in the solubility of the gluten proteins in 10% sodium salicylate among the different flours, the proteins of the lower quality stream apparently having no greater solubility in this medium than the best flours. Similarly, the quantity of protein fractionated by MgSO_4 does not differ markedly from sample to sample in this series. This fraction, when computed as percent of flour, appears to vary significantly with flour strength, but this is caused by differences in the protein or gluten content of the flours, as the gluten fraction in the flour is largely influenced by the percent of crude protein present. Large differences in the starch resistivity are evident. These values are extremely low for the first two sizing flours, which were high in diastatic activity. It would appear accordingly that the high saccharogenic activity of these flours is due largely to a lower starch resistance to hydrolysis rather than to an increase in enzymic activity. The same reasoning applies to the duster flour. It is probable that the starch granules in this sample had been injured by severe grinding and mechanical treatment in the mill with consequent impairment of resistance to hydrolysis by the diastatic enzymes. The Standard Basic Method did not differentiate as sharply among the flours in respect to loaf volume as the malt-phosphate-bromate baking. The latter method brought out in a striking manner the potential baking quality of the first five break flours. This reaction corresponds essentially to the results obtained by other workers and already cited in this paper. The break flours produced loaves of darker color and lower texture rating than the better middlings flours. Loaf volume data only were included in Table II, as this characteristic of the baked loaf is subject to quantitative measurement.

The statistical constants computed from the data are shown in Table III. The range in diastatic activity and starch resistivity is extremely large, reflecting significant differences in these attributes from sample to sample. Flour ash is also extremely variable, as would be expected in material of this nature. Large variations are evident in flour protein, crude wet gluten, and malt-phosphate-bromate loaf volume. The quantity of protein fractionated, and the Standard Basic loaf volume, on the other hand, have relatively small variability.

The correlation coefficients calculated from results of the various determinations are presented in Table IV, with tests of significance. Relatively high positive correlations are shown between loaf volume and flour protein, loaf volume and wet crude gluten, and loaf volume and gluten protein in the flour. No significant correlation was demonstrated between loaf volume and the protein fraction. This

TABLE II
PROTEIN FRACTIONATION DATA, STARCH RESISTIVITY, AND LOAF VOLUME
OF THE MILL-STREAM FLOURS

Serial number	Soluble protein mg. per 100 c.c.	Protein removed by MgSO_4 per 100 c.c.	Protein removed by MgSO_4 as percent of flour	Starch resistivity	Loaf volume (13.5% moisture basis)	
					Standard basic	Malt-phosphate-bromate
		Mg.	%		C.c.	C.c.
37-11- 4	490	134.0	3.20	751	568	790
5	481	139.6	3.59	685	566	840
6	475	137.4	4.33	605	584	960
7	478	148.2	5.05	599	581	920
8	496	151.0	4.92	579	566	825
9	479	148.8	3.25	445	538	634
10	479	139.0	3.31	442	552	706
11	450	126.0	2.60	356	560	650
12	473	141.4	2.93	249	544	560
13	476	112.2	2.27	515	526	565
14	481	148.2	2.99	324	522	555
15	473	133.8	2.70	416	520	550
16	496	152.6	3.10	324	518	605
17	504	156.0	3.32	385	536	605
18	485	147.0	3.44	360	555	710
19	484	158.4	3.94	509	548	725
20	496	157.2	3.82	342	562	739
21	499	155.6	3.76	463	548	704
22	484	149.8	3.51	377	534	718
23	464	140.2	3.80	533	555	715
24	467	139.0	3.36	390	552	692
25	499	156.6	3.75	380	532	660
26	487	139.0	3.45	306	532	674
27	487	140.8	3.45	435	553	702
28	473	134.4	2.59	235	532	535
29	493	159.0	4.47	477	595	750
30	493	149.4	4.41	501	585	746

TABLE III
STATISTICAL CONSTANTS CALCULATED FOR THE VARIOUS DETERMINATIONS

Determination	Mean	Minimum	Maximum	Standard deviation	Coefficient of variability
Crude flour protein, %	14.1	11.8	18.9	1.96	13.90
Wet crude gluten, %	48.8	38.6	68.2	7.73	15.84
Gluten protein in flour, %	12.6	10.3	16.9	1.80	14.29
Protein removed by MgSO_4 , mg.	144.2	112.2	159.0	11.29	7.83
Diastatic activity, mg. maltose	252.1	135.8	418.4	65.14	25.84
Starch resistivity	443.8	235	751	123.68	27.87
Flour ash, %	0.69	0.34	1.49	.330	47.80
Protein solubility in sodium salicylate, mg.	483.0	450	504	94.7	19.61
Loaf volume (Standard Basic), c.c.	550.5	518	584	20.55	3.73
Loaf volume (malt-phosphate-bromate), c.c.	697.59	550	960	105.84	15.17

fraction appears to be relatively constant in the gluten of flours originating in various parts of the wheat kernel. A negative relationship is apparent between malt-phosphate-bromate loaf volume and diastatic activity. This is evidently not due to the higher saccharogenic activity of low grade flours, as no significant correlation was found between diastatic activity and flour ash. A positive relationship between loaf volume and starch resistivity was noted. Protein solubility had no connection with loaf volume. A high positive correlation existed between flour protein and wet crude gluten as would be expected, while high protein millstream flours tend to be also high in ash. No relation was evident between flour protein and gluten protein solubility or between flour protein and the quantity of gluten protein fractionated by MgSO_4 .

TABLE IV
CORRELATION COEFFICIENTS COMPUTED FROM THE DATA

Variables correlated		r_{xy}	P^1
X	Y		
Loaf volume (Standard Basic)	Crude flour protein	+ .7236	<.0001
Loaf volume (malt-phosphate-bromate)	Crude flour protein	+ .7928	<.0001
Loaf volume (Standard Basic)	Wet crude gluten	+ .7829	<.0001
Loaf volume (malt-phosphate-bromate)	Wet crude gluten	+ .8866	<.0001
Loaf volume (Standard Basic)	Gluten protein in flour, %	+ .7762	<.0001
Loaf volume (malt-phosphate-bromate)	Gluten protein in flour, %	+ .8845	<.0001
Loaf volume (Standard Basic)	Protein removed by MgSO_4 (mg.)	+ .2539	.2022
Loaf volume (malt-phosphate-bromate)	Protein removed by MgSO_4 (mg.)	+ .2118	.2908
Loaf volume (Standard Basic)	Diastatic activity	- .3063	.1222
Loaf volume (malt-phosphate-bromate)	Diastatic activity	- .6982	.0001
Loaf volume (Standard Basic)	Starch resistivity	+ .5786	.0002
Loaf volume (malt-phosphate-bromate)	Starch resistivity	+ .7276	<.0001
Loaf volume (malt-phosphate-bromate)	Protein soluble in sodium salicylate, mg.	+ .1457	.4668
Crude flour protein, %	Wet crude gluten	+ .9522	<.0001
Crude flour protein, %	Flour ash	+ .7422	<.0001
Crude flour protein, %	Protein removed by MgSO_4 (mg.)	+ .3649	.0611
Crude flour protein, %	Protein soluble in sodium salicylate, mg.	+ .0389	>.5485
Diastatic activity	Starch resistivity	- .7429	<.0001
Diastatic activity	Flour ash	+ .1411	.4846

¹ P = probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.

Tests of the significance of the differences between several of the correlation coefficients are listed in Table V. The transformation of the correlation coefficient r to the Z value was performed, as recommended by Fisher (1928), and the significance of the difference $Zr_1 - Zr_2$ was then evaluated in terms of the probability of the observed difference arising through sampling errors. The relative deviate, X^1 , is found from the ratio of the difference between the Zr values and the standard deviation of the difference

$$\left(\sqrt{\frac{1}{N_1 - 3} + \frac{1}{N_2 - 3}} \right)$$

TABLE V
TESTS OF THE SIGNIFICANCE OF THE DIFFERENCE BETWEEN
CORRELATION COEFFICIENTS

Correlation coefficient	Zr	$d/\sigma d$	X^1	P^1
.7928	1.0752	0.1601	0.55	>.5485
.7236	0.9151	<u>.2887</u>		
$d = (\text{diff.})$	0.1601			
.8866	1.4062	.3533	1.22	.2229
.7829	1.0529	<u>.2887</u>		
$d = (\text{diff.})$	0.3533			
.7829	1.0529	.1378	0.48	>.5485
.7236	0.9151	<u>.2887</u>		
$d = (\text{diff.})$	0.1378			
.8866	1.4062	.3310	1.15	.2508
.7928	1.0752	<u>.2887</u>		
$d = (\text{diff.})$	0.3310			
.7276	0.9230	.2626	0.91	.3631
.5786	0.6604	<u>.2887</u>		
$d = (\text{diff.})$	0.2626			

¹ P = probability of the observed difference arising through errors of random sampling.

There are no significant differences demonstrated among the five pairs of correlation coefficients shown in the table. Accordingly, the coefficients obtained between the malt-phosphate-bromate baking results and flour protein, and between malt-phosphate-bromate values and wet crude gluten, are not significantly higher than the corresponding values obtained with the Standard Basic results. Similarly, the correlation yielded by loaf volume and wet crude gluten is not significantly higher than the correlation between loaf volume and flour protein. No significant difference existed between the relations between starch resistivity and the loaf volumes registered by the two baking methods. It is therefore apparent that the use of an "improver" formula did not give a significant increase in information as compared with the Standard Basic Method in this set of millstream flours. This conclusion is in agreement with the results obtained by the senior author when working with Canadian hard red spring wheat mill-stream flours. A knowledge of flour protein yields as accurate information in regard to baking value, as registered by loaf volume, as a determination of wet crude gluten in these mill-stream flours.

As it appeared possible that the observed negative correlation between diastatic activity and loaf volume might be due in part at least to the influence of starch resistivity, partial correlation technique was employed to eliminate, first, the effect of diastatic activity upon the loaf volume—starch resistivity relationship. A positive relation-

ship between the two variables was obtained under these conditions. The effect of diastatic activity upon loaf volume when starch resistivity was held constant was also determined, and a relatively small negative correlation coefficient obtained. At least part of the relation between diastatic activity and loaf volume is apparently due to lowered starch resistance to diastatic action. This is especially true of flours coming from the germ reductions and the dust reel. These correlations are shown in Table VI.

TABLE VI
PARTIAL CORRELATIONS

Variables correlated	Variable held constant	Correlation coefficient
Starch resistivity and loaf volume (malt-phosphate-bromate)	Diastatic activity	+ .4359
Diastatic activity and loaf volume (malt-phosphate-bromate)	Starch resistivity	-.3433

Table VII shows the method of predicting the percent of wet crude gluten in these flours from a knowledge of the flour protein content. The determination of crude gluten by the method of washing out the flour starch and other constituents as far as possible was considered to be very important at one time before the Kjeldahl test or its modifications came into general use for determining flour protein.

TABLE VII
PREDICTION OF PERCENT OF WET CRUDE GLUTEN FROM PERCENT
OF CRUDE FLOUR PROTEIN

Linear regression coefficient
Percent of wet crude gluten ^(y) on percent of crude flour protein ^(x) $B_{xy} = 3.942$
Linear regression equation
Estimation of percent of wet crude gluten (y) from percent of crude flour protein (x) $y = -6.78 + 3.94x$ Standard error of estimate = 2.4%

Summary and Conclusions

A series of 27 mill-stream flours were analyzed and baked by two methods—the Standard Basic, and the malt-phosphate-bromate. Wet crude gluten was also washed from the flours, employing a 0.1% sodium phosphate solution whose pH was approximately 6.8. These glutes were then dispersed in 10% sodium salicylate solution and fractionated by the addition of a small quantity of $MgSO_4$. The data obtained appeared to justify the following conclusions.

1. The use of malt-phosphate-bromate does not yield significantly higher correlations with various quality factors than are obtained with the Standard Basic Method. It does, however, give greater differ-

entiation in regard to loaf volume between the samples, and brings out the potentialities of high gluten break flours in a striking manner. Its incorporation in methods for testing mill-stream flours produced from hard red spring wheat would therefore seem advisable.

2. The quantity of gluten protein removed from a sodium salicylate dispersion by a single small addition of MgSO_4 does not appear to vary appreciably from sample to sample in mill streams, regardless of the quality of the flour, and is not related to any appreciable degree with other factors of quality. The quantity of this constituent present in the gluten washed from flours originating in different portions of the wheat kernel therefore must be fairly constant. As it will vary in the flour according to the gluten content when calculated as percent of the flour, this fraction is highly and positively related to loaf volume.

3. The better quality flours appeared to have lower diastatic activity in these samples. A part of this effect at least was due to differences in the resistivity of the starch substrate to diastatic attack. Flours presumably containing germ particles exhibited high diastatic activity coupled with lower starch resistivity. It is probable that in one instance severe grinding had the effect of lowering starch resistance to injury to the starch granule, thus increasing its susceptibility to diastatic action.

4. Flour protein appeared to be as highly related as wet crude gluten percentage to loaf volume in this series of flours, thereby justifying the substitution of the Kjeldahl test for the gluten washing determination. Flour protein, as well as wet crude gluten and gluten protein, was high in the break flours. Flour protein tended to increase with ash content; that is, these two constituents appeared to be related to each other in their location in the wheat kernel.

Literature Cited

- American Association of Cereal Chemists
1935 Cereal Laboratory Methods, Third Edition. Published by the A. A. C. C.
Bailey, C. H.
1925 The chemistry of wheat flour. The Chemical Catalog Co., New York.
Blish, M. J.
1928 Standard experimental baking test. Cereal Chem. 5: 158-161.
— and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. Cereal Chem. 10: 189-202.
Fisher, R. A.
1928 Statistical methods for research workers. Published by Oliver and Boyd, Edinburgh.
Harris, R. H.
1930 A study of commercially milled flours dealing with protein and its relation to peptization and baking strength. Cereal Chem. 8: 113-133.
1937 A study of gluten protein fractionation from sodium salicylate solution. Part I. Durum wheat gluten fractionation. Cereal Chem. 14: 695-707.
1938 A study of gluten protein fractionation from sodium salicylate solution. Part II. Bread wheat gluten fractionation. Cereal Chem. 15: 80-90.

- and Sanderson, T.
 1938 A comparison between the Standard Basic and malt-phosphate-bromate baking procedures on North Dakota wheats. *N. Dak. Agr. Exp. Sta. Cir.* 61.
- Jacobs, B. R.
 1915 Report to the American Society of Milling and Baking Technology on collaborative studies. Original not seen.
- Malloch, J. G.
 1929 Studies on the resistance of wheat starch to diastatic action. *Can. J. Research* 1: 111.
- Pascoe, T. E., Gortner, R. A., and Sherwood, R. C.
 1930 Some comparisons between commercially and experimentally milled flours. *Cereal Chem.* 7: 195-221.
- Rich, C. E.
 1934 The chemical and physico-chemical changes induced in wheat flour by artificial maturing agents. *Cereal Chem.* 11: 201-227.
- Richardson, Clifford
 1884 An investigation of the composition of American wheat and corn. *U. S. Dept. Agr. Bur. Chem. Bull.* 4.
- Shellenberger, J. A., and Bailey, C. H.
 1936 Biochemical distinctions between barley varieties. *Cereal Chem.* 13: 631-655.
- Swanson, C. O., Willard, J. T., and Fitz, L. A.
 1915 Kansas Flours. *Kans. Agr. Exp. Sta. Bull.* 202.
- Weaver, H. E.
 1921 The relation of ash and gluten in wheat flour. *J. Am. Assoc. Cereal Chemists* 6, No. 2: 11-13.

DIRECT CURRENT OF STABILIZED VOLTAGE FOR THE TAG-HEPPENSTALL MOISTURE METER AND OTHER LABORATORY INSTRUMENTS¹

EARL B. WORKING

Kansas Agricultural Experiment Station, Manhattan, Kansas

(Received for publication January 26, 1937)

Numerous instruments requiring direct current at potentials above 40 volts are coming into routine laboratory use. In the past these have usually been supplied with current by dry batteries, but a tendency is now growing towards a rectified supply from the alternating current power lines. This is urged not only by the cost of periodic replacements of the batteries, but also by their gradual failure in voltage and their occasional failure at inconvenient times.

Various types of rectifier circuits and voltage control units have been developed for different radio uses, but if the most satisfactory and at the same time economical circuit is to be developed, the current requirements of the instrument to be supplied must first be studied. Certain photoelectric devices, electronic voltmeters and other instruments have a low and nearly constant current demand, which simplifies

¹ Contribution No. 54, Department of Milling Industry, Kansas Agricultural Experiment Station.

the requirements unless a very high degree of voltage constancy is required. If, however, the current drain varies widely, the rectifier design must be carefully planned or the voltage will drop seriously as the current increases.

The Tag-Heppenstall moisture meter has been found very useful for rapid determinations of moisture in grain where high accuracy is not essential. This meter requires a battery of 112.5 volts, and as used at this laboratory, replacements are necessary at least twice a year. Since the cost of these amounts to a considerable sum over a period of years, it seemed desirable to construct a rectifier to operate from the power line, and the study was continued to include an electronic voltage regulator for use with instruments requiring very stable voltage, or in cases where the voltage of the power supply fluctuates widely.

A study of the circuit of the Tag-Heppenstall meter shows that while it uses a current of only about 1 microampere when testing wheat of 8% moisture content, the current is nearly 15 milliamperes for wheat of about 24% moisture, the maximum for which the meter is designed. A modification of the meter recommended by Coleman and Fellows (1936) for increasing the range to include grain of higher moisture content increases the possible current consumption to 20 milliamperes at 90 volts, the limit set by the protective resistor of the meter. This full range is not usable as it corresponds to a short circuit of the electrodes, but the rectifier circuits described here have been required to deliver this current within the permissible voltage drop.

Accuracy Required

The permissible voltage variation may be estimated from the voltage drop allowed by the manufacturers in their instructions for testing batteries for satisfactory strength. The meter is standardized by switching to a tap marked *S* which connects to a standard resistor drawing about 1.5 milliamperes. A rheostat is then adjusted to bring the meter to a reading of 50. To check the condition of the batteries the switch is then changed to a tap marked *T*, connected with a standard resistor drawing about 13.5 milliamperes. Instructions are that if the meter reading drops below 48 on this tap the battery should be replaced. Assuming a voltage of 100, which is nearly the minimum useful voltage of the 75 cell battery used, this would apparently correspond to a voltage drop of 4 volts, but it actually corresponds to 6 volts since the standardizing resistors are so chosen that if the voltage does not drop, the meter reading rises to 51. Judging from this allowance, it was assumed that for a rectifier supply circuit a drop of

3 volts at the maximum current consumption would be well within the requirements for accuracy.

However, a more fundamental measure of the allowable voltage fluctuations may be obtained from the standard error of the instrument. The error of replication of the Tag-Heppenstall meter is very low, but since the electrical conductivity of wheat is not perfectly correlated with moisture content, the error of its prediction of moisture content is considerably higher. Cook, Hopkins, and Geddes (1934) report an observed standard error of prediction of 0.21% moisture in testing hard red spring wheats below 17% in moisture content using as a standard the vacuum oven with a drying period of 16 hours. For wheat from 17 to 24% in moisture content the observed standard error was 0.58% moisture, or nearly three times as much as with the drier wheat.

Coleman (1931) found an average deviation from air-oven tests of 0.17% as measured on 147 samples of hard red winter wheat, with 13 samples in error by 0.4% or more. However, Cook, Hopkins, and Geddes (1934) found the errors for the air-oven method greater than those for the Tag-Heppenstall meter, except for wheat moisture content above 17%.

Measurements made in the laboratories of the Milling Department of Kansas State College in comparison with the results given by the steam-shelf vacuum oven described by Anderson (1936) show that the error of the Tag-Heppenstall meter varies considerably from year to year. Excluding all samples which had been artificially wetted, since the error for these was found to be greater, the standard error was 0.29% moisture for the crop of 1935 and 0.49% for the crop of 1936. For the former the errors were about evenly divided, with a majority negative; for the latter year not only was a large majority of the errors positive, but the negative errors averaged as great as those of the preceding year. For these two years the samples not artificially wetted were all below 11% in moisture content, or lower than most of the samples tested by Cook, Hopkins, and Geddes (1934). The greater error is possible due to the fact that many varieties and different growing conditions were included.

From the accuracy of the results obtained from the meter in this laboratory it was judged that voltage fluctuations were unimportant if the resulting error in moisture indication was not over 0.1%, but considering the greater accuracy reported by other investigators, it was decided to limit the permissible variation to 0.05% moisture on all samples of 17% moisture or less. This appears very conservative, considering that for the vacuum-oven method, the most accurate moisture method available, Cook, Hopkins, and Geddes (1934) found a standard error of 0.118% moisture.

Since the accuracy of any grain moisture determinations, and particularly those made by the Tag-Heppenstall moisture meter, decreases rapidly at moisture contents above 17%, the theoretically permissible variation was allowed to increase gradually to 0.1% at 19% moisture content, 0.3% at 21% moisture, and 0.5% at the maximum reading of the instrument with the additional resistor recommended by Coleman and Fellows (1936).

Assuming these errors to be negligible, the permissible voltage fluctuation is plus or minus 4 volts or more at any point on the scale. This is a considerably more stringent requirement than that placed on the use of old batteries by the instructions of the manufacturer.

Simple Circuit

Probably the most easily assembled circuit falling within the above requirements is that shown in Figure 1 using the type 874 R.C.A.

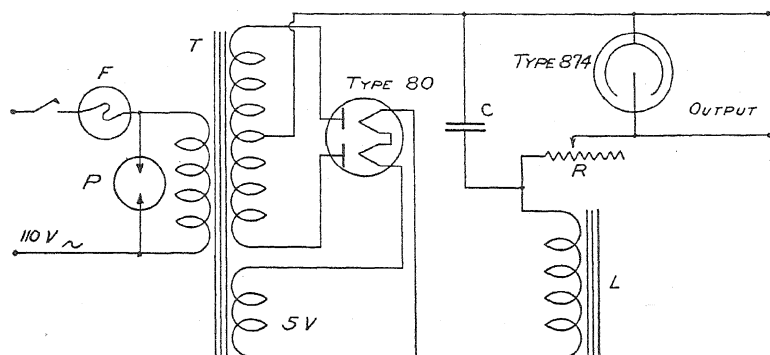


Figure 1. C—condenser, 8 mfd.; F—fuse, 1 ampere; L—choke, 11-henry, 65 milliamperes; P—neon lamp, $\frac{1}{4}$ watt; R—adjustable resistor, 6,000 ohms, 25 watts; T—power transformer, 50 milliamperes, about 340 volts each side of center tap.

gas discharge voltage regulator tube. The power transformer and choke may be inexpensive units as made for midget radio sets, but with current ratings of at least 50 milliamperes. When the circuit is assembled, a milliammeter should be connected in series with the type 874 tube and resistor R adjusted to give a current of 35 or 40 milliamperes when no current is being drawn from the output. Under these conditions voltage fluctuations up to 15% in the supply current will cause no appreciable change in the output voltage. A current drain of 20 milliamperes causes a voltage drop of about two volts, or only about half that permitted for the Tag-Heppenstall meter.

If for any reason it should be desirable, the amount of this voltage drop may be somewhat reduced by using a power transformer delivering about 130 volts each side of the center tap, and a type 83 or 82

tube instead of the type 80. The choke should then be of about 30 henries inductance, and the adjustable resistor may be rated at about 1,000 ohms, 10 watts. With this change, when the output voltage begins to drop there is a considerable increase in the current delivered by the rectifier. The output voltage fluctuation due to changes in the input voltage will be somewhat increased.

Adjustable Voltage

However, in this laboratory it was desired to experiment with voltages as low as 25 volts for wheats of high moisture content and as high as 160 volts for wheats of low moisture content. Obviously the circuit just described would not furnish such voltages, so the circuit shown in Figure 2 was used. It is apparent that the output

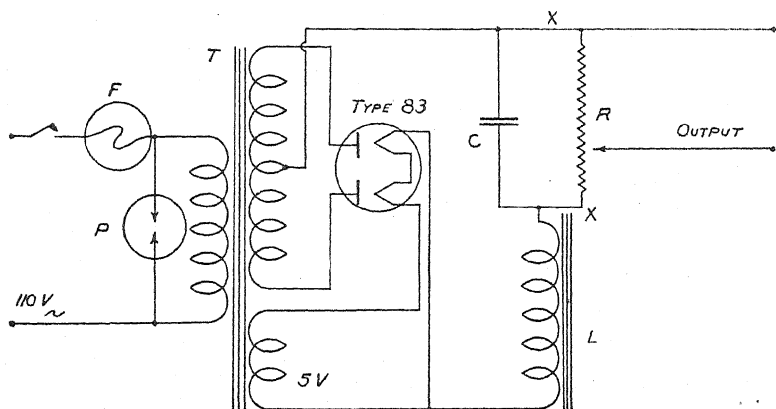


Figure 2. C—condenser, 8 to 16 mfd.; F—fuse, 1 ampere; L—swinging choke, 8–30 henry at 250–0 milliamperes; P—neon lamp, $\frac{1}{4}$ watt; R—adjustable resistor, 1,000 ohms, 200 watts; T—power transformer, 225 milliamperes, 200 volts each side of center tap; X, X'—points for substitution of output shown in Figure 2A.

voltage will drop as the current drain increases, and the following precautions should be observed to minimize this effect: The power transformer should operate at fairly near magnetic saturation, and should deliver a voltage as low as will supply the required output voltage. The rectifier tube should preferably be of the mercury vapor type, though the 83 V tube gives satisfactory results. A choke of fairly high inductance should form the filter input. The current flowing continuously through voltage divider R should be as high as practicable.

As assembled from readily available and inexpensive radio parts according to the specifications given, a voltage drop of 3 volts is observed at a drain of 20 milliamperes, the maximum consumption of the Tag-Heppenstall meter. When tested with the moisture meter

itself according to the method described for testing batteries, the reading drops only $\frac{1}{3}$ scale division when the meter switch is changed from tap *S* to tap *T*, instead of 2 scale divisions, the limit set by the manufacturers.

This circuit does not compensate for line voltage fluctuations beyond the regulation characteristics of the power transformer used. This has been satisfactory here as the line voltage is relatively constant, and four-volts fluctuation in input at 220 volts causes only one volt change in output at 100 volts. If occasional voltage fluctuations of considerable magnitude must be guarded against, the addition of a voltmeter, *V*, and a readily adjustable power rheostat, *R*₂, as shown in Figure 2A, forms a satisfactory solution. If the moisture meter is to be operated only at normal voltage, the power transformer may be selected to deliver about 130 volts each side of the center tap, *R*₁ may

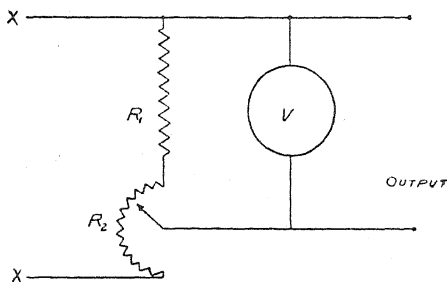


Figure 2A. *R*₁, *R*₂—resistor and power rheostat (potentiometer connection) specifications as required by the power transformer used; *V*—voltmeter, full scale reading equal to maximum voltage to be drawn from circuit.

be rated at 500 ohms, 100 watts, and *R*₂ at 100 ohms, 25 watts. *V* may be an inexpensive meter of 100 volt range; its accuracy need not be high but the scale should be long enough that fluctuations over two or three volts may be readily seen and corrected by a change in *R*₂. This adjustment may easily be made while the moisture meter is in operation if the motor-driven model is used, and as previously noted, fluctuations are insignificant unless they exceed four volts. With the power transformer delivering only 130 volts, the voltage drop at the meter at its maximum current consumption is only two volts, too little to require readjustment of the rheostat.

Electronic Voltage Regulator

In order to furnish a power supply of highly accurate voltage for comparative testing, the circuit shown in Figure 3 was designed and assembled. This is a modification of a circuit designed by R.C.A. for their type 874 voltage regulator tube. The latter circuit could not

be used since the voltage of the regulator tube plus the necessary bias for the power tube gives an output voltage too high for the Tag-Heppenstall meter. The ordinary 2-watt neon glow lamp with protective resistance removed from the base gives very satisfactory results as a regulator, though its voltage varies more than that of the type 874 for a given change in operating current. The screen of the type 57 tube is ordinarily connected to a point on R_1 with a potential about 80 volts positive to the cathode, the exact setting depending on individual circuit characteristics. If the output voltage increases with increased current drain, lower the screen voltage, and *vice versa*.

The specifications given are for inexpensive parts satisfactory for delivering up to 20 milliamperes at about 100 volts. Table I gives the maximum range of variation of input voltage over which output voltage will be accurately regulated for any current drain from 0 to 20 milliamperes: The output is adjusted to the voltage given, or any

TABLE I

PERMISSIBLE INPUT VOLTAGE FLUCTUATION FOR ACCURATE VOLTAGE REGULATION
AT ANY CURRENT DRAIN, 0 TO 20 MILLIAMPERES

Input voltage, type 2A3 tube	Input voltage, type 45 tube	Output voltage adjusted to
82 to 130	90 to 120	100 volts
85 to 150	90 to 125	105 "
87 to 150		110 "

intermediate value, by the potentiometer R_5 . Higher voltages are available at current drain lower than 20 milliamperes. Except at the extremes of this range, output voltage fluctuations should not be discernible on a 150-volt meter with a 5-inch scale. This has been found satisfactory as current source for a photoelectric colorimeter and for a high impedance vacuum-tube voltmeter.

If currents much larger than 20 milliamperes are to be drawn, or if higher output voltages are desired, the power transformer should deliver a higher voltage, and the transformer, rectifier tube, and chokes should have current capacities with ample reserve above the expected drain. It would be well to increase the resistance of the bleeder system, R_3 - R_4 - R_5 , and in any case R_1 should be so chosen as to pass approximately 10 milliamperes through N . The type 45 tube will not be satisfactory for currents above about 30 milliamperes, but the 2A3 will easily deliver 50 milliamperes, and for higher currents the circuit may be adapted to use the 6L6 tube, or two of them in parallel,

In Figure 3 the regulating glow lamp *N* may be so mounted as to serve as a pilot lamp, therefore no other pilot has been shown. In Figures 1 and 2 an alternative to the neon pilot lamp is a 6.3-volt, 0.15-ampere radio panel light connected across the 5-volt filament winding of the power transformer. At this reduced voltage the bulb will draw very little current and last almost indefinitely.

Summary

The current requirements of the Tag-Heppenstall moisture meter are studied and two rectifier circuits are shown which fulfill them. The second circuit permits adjustment of its output voltage over a wide range. A third circuit is shown incorporating an electronic regulator which will maintain the output voltage constant within 0.1 volt or less over a wide range of input voltages at any current drain within the capacity of the power tube.

Literature Cited

Anderson, J. E.

- 1936 Some facts concerning vacuum-oven moisture determination. *Cereal Chem.* 13: 437-452.

Coleman, D. A.

- 1931 Moisture determinations in wheat with special reference to quick methods. *Cereal Chem.* 8: 315-335.

— and Fellows, H. C.

- 1936 Handbook of Instructions for the Installation and Operation of the Tag-Heppenstall Moisture Meter. U. S. D. A. Bur. Agr. Economics. U. S. G. S. A.—MBI—1.

Cook, W. H., Hopkins, J. W., and Geddes, W. F.

- 1934 Rapid determination of moisture in grain. II. *Can. J. Research* 11: 264-289; 409-447; 547-563.

THE RELATIONSHIP BETWEEN DIASTATIC ACTIVITY (MALTOSE FIGURE) AND "GASSING POWER" OF EXPERIMENTALLY MILLED FLOURS FROM SOME AUSTRALIAN WHEATS

R. A. BOTTOMLEY

W. S. Kimpton & Sons, Melbourne, Victoria, Australia

(Received for publication December 22, 1937)

Introduction

The importance of the diastatic activity of flour is too well recognised to need stressing here. Commercially, increasing control is being carried out both by the addition of malted wheat flour and by careful wheat blending. This necessitates accurate and, if possible, quick methods of analysis. Several methods are available for the determination of diastatic activity and gassing power from the point of routine mill control, but the selection of any one is a matter of choice, or circumstance, and opinions still differ with regard to their relative merits.

In this laboratory the routine method used for the determination of diastatic activity or maltose figure is Kent-Jones' modification of Rumsey's method while the Brabender Fermentograph is used for the determination of "gassing power." However, it was found over a period of 18 months that the gas production of a dough, as judged by the diastatic activity so determined, frequently failed to correspond with that shown by the Fermentograph.

This experiment was, therefore, carried out with the object of examining the relationship between the above two methods, though later the determination of diastatic activity by the Blish-Sandstedt method was included for comparative purposes.

Methods

1. DIASTATIC ACTIVITY

(a) *Kent-Jones' modification of Rumsey's method.* This method, as described by Kent-Jones (1927), was followed except that 15 instead of 30 g. of flour were used per test, and water, acid, *etc.* were reduced in proportion. Briefly, the method consists of digesting an unbuffered flour suspension for 1 hour at 27° C., then clarifying and preventing further diastatic activity by means of sodium tungstate solution and

sulphuric acid. The suspension is next filtered and the clear filtrate is titrated against Fehling's solution, using methylene blue as an internal indicator.

(b) *Blish-Sandstedt method.* This method is widely used for routine work and was tentatively recommended as a standard method by the conference of (Government) cereal chemists held in Sydney, December 1936. According to Davis and collaborators (1937) the method is about as satisfactory as the Schoorl and Bertrand methods for measuring reducing sugars after diastasis. It was, therefore, included in this experiment for comparative purposes and was used as set out in the A.A.C.C. *Cereal Laboratory Methods* (1935). (See also Davis and Worley, 1934.) In this method buffered flour suspensions are digested at 30° C. for 1 hour, after which the diastatic activity is inhibited as before with sodium tungstate solution and sulphuric acid. An aliquot of the filtrate is next treated with excess standard potassium ferricyanide, the unreduced ferricyanide being determined by titration with standard sodium thiosulphate.

2. "GASSING POWER"

The gassing power was measured by the Brabender Fermentograph in the usual way. That is, the flour, yeast, salt and water dough was made in the Farinograph mixing bowl by mixing until the maximum dough development had been reached at the 600 unit line, usually about 4 minutes. Four hundred grams of the dough were then quickly weighed off, transferred to the Fermentograph balloon, fitted to the recording device and immersed in the water bath at 30° C. By knocking back the dough at the end of each hour and allowing the carbon dioxide produced during fermentation to escape, it was possible to measure the hourly rate of production of CO₂. Fermentation was continued until the rate had fallen to a low figure compared with the maximum or until 7 hours had elapsed.

The only question that arises in this test is the selection of the yeast and salt quantities. Several formulae were tried but the one finally adopted was that recommended by Brabender, *i.e.* 2 $\frac{2}{3}$ % yeast and 1 $\frac{3}{8}$ % salt, with which the gas produced approximately corresponds to that formed in a 3-hour dough (4 hours from dough to oven).

Selection and Preparation of Samples

Since we are primarily concerned with controlling the diastatic activity and other properties of the flour by means of wheat blending, the consideration of experimental mill flours is of paramount importance. The flours used in this test were, therefore, experimentally milled flours and it was decided to examine those from some wheat

varieties known to differ in their diastatic activity and also some about which a certain amount of controversy still obtains.

Ten-pound samples of wheat (1936-37 season) were supplied by various official organisations and were definitely *bona fide* samples of the stated variety. They are listed below together with the comments received with them.

Laboratory number	Sample	Source of supply and comments
1	Ranee 4H. Vic.	Dookie Agricultural College—experimental area.
2	Dundee. Vic.	
3	Nabawa. N.S.W.	New England Experiment Farm Glenn Innes. This sample is not from one plot, but from 5 replications.
4	Baringa. N.S.W.	Agricultural Department N.S.W. "Each sample is made up of good typical samples from a number of districts and may be taken as quite representative of the varieties."
5	Pusa. N.S.W.	
6	Ranee 4H. S.A.	Turretfield Seed Farm
7	Ford. S.A.	
8	Nabawa. S.A.	
9	"Selection 28." S.A.	
10	Ford. S.A.	Roseworthy Agricultural College
11	Ranee. S.A.	
12	Nabawa. W.A.	Wongan Hills Research Station. "Soil is very sandy and gravelly and hence wheats are low in protein. In the 1935-36 season 30.7% of the total acreage sown to wheat in Western Australia was Bencubbin and 8% sown to Nabawa."
13	Bencubbin. W.A.	

After examination 3,000 g. of each sample were conditioned and then ground in the Brabender experimental mill. Conditioning varied slightly with the wheat, the harder wheats such as the Pusa sample being given a little more water than the others, but in each case the samples were conditioned overnight with sufficient water to bring their moisture content to 15%. Then in most cases, $\frac{1}{2}$ to 2% of water was added to the sample $\frac{1}{2}$ to 1 hour before grinding. The variation in conditioning was made in order to produce within as narrow limits as possible flours with a moisture content of 15.0% and an extraction of approximately 58%. The flours were stored in air-tight tins throughout the experiment.

The flours were subjected to gluten, protein, moisture and ash tests besides the diastatic activity determinations, and their water absorption and strength curves were determined by the Farinograph. In order that a definite idea of the samples under consideration may

be obtained, the results of these analyses are given in Table I, where the samples have been tabulated in order of their strength as judged from their analysis and their Farinograph curve.

It is naturally difficult to compare such widely differing varieties as Baringa and Ranee, as has been done above, since the question as

TABLE I
SAMPLES ARRANGED IN ORDER OF STRENGTH (ANALYTICAL FIGURES BASED ON FLOUR OF 15.0% MOISTURE CONTENT)

Sample	Extraction	Ash	Water absorption	Dry gluten	Protein (N X 5.7)	Comments
Pusa. N.S.W.	% 53.9	% 0.716	% 68.0	% 16.8	% 13.08	Strong, hard wheat. Very good sample.
Ford. S.A. (R)	61.7	0.518	58.0	15.8	11.54	Medium strength wheat, stability not so good as with Pusa sample.
Baringa. N.S.W.	55.2	0.756	71.3	18.4	14.19	Medium strength. Hard vitreous grains, stability low for this protein content.
Dundee, Vic.	59.3	0.585	66.0	11.8	9.56	Medium—nearly 50% mottled grains.
Nabawa. N.S.W. (G.I.)	61.8	0.440	59.6	10.1	8.61	Medium—very good stability for such a low-protein figure.
Ranee. S.A. (R)	63.6	0.442	60.1	11.7	9.43	Weak but good. Excellent sample of Ranee wheat.
Ford. S.A. (T)	58.2	0.430	54.5	10.4	8.95	Weak but good. Very stable but does not compare with sample from Roseworthy.
Ranee 4H. S.A. (T)	58.1	0.464	59.2	10.1	8.60	Weak. Grains very slightly bleached.
Nabawa. S.A. (T)	55.3	0.500	52.0	8.4	7.34	Weak, but very good stability.
Bencubbin. W.A.	58.7	0.524	57.8	10.6	8.68	Weak. Grains extremely long and plump.
Ranee 4H. Vic.	56.0	0.436	59.3	10.0	8.55	Weak. Sample had a good appearance.
"Selection 28." S.A. (T)	54.1	0.540	55.0	9.4	8.14	Weak—average sample.
Nabawa. W.A.	63.3	0.522	59.0	7.0	7.01	Very poor with practically no stability. Milled very well.

to whether one is or is not "better" than the other depends to a large extent on the individual requirements of the miller. However, considering samples of the one variety it may be seen that they fall in the following order in regard to "strength:"

- | | | |
|---|--|---|
| <p>Ranee</p> <ol style="list-style-type: none"> 1. Roseworthy, S.A. 2. Turretfield, S.A. 3. Dookie, Vic. 4. "Selection 28." Turretfield, S.A. | <p>Nabawa</p> <ol style="list-style-type: none"> Glenn Innes, N.S.W. Turretfield, S.A. Tongan Hills, W.A. | <p>Ford</p> <ol style="list-style-type: none"> Roseworthy, S.A. Turretfield, S.A. |
|---|--|---|

The "Selection 28" sample has been placed with the Ranee samples because of the similarity of their characteristics.

It should be noted here that the samples of Bencubbin and Nabawa wheats from Western Australia are not representative as regards "strength" of the wheat grown in that State.

Relationship between Experimentally Milled Flours from the Brabender Mill and Corresponding Commercial Flours

Unlike the flour produced by the majority of experimental mills, that from the Brabender mill has a diastatic activity (as measured by above methods) considerably higher than the corresponding commercial flour. The relationship varies with the type of wheat ground, the difference being greater with harder than with soft wheats. On average, with soft wheats, such as the Ranee samples mentioned above, the diastatic activity as measured by the Kent-Jones method is from 20 to 40 mg. per 10 g. flour lower in the commercial than in the corresponding experimental flour. With harder wheats, such as Dundee, the difference may vary from 100 to 140 mg. per 10 g. of flour. The differences in gassing power as determined by the Fermentograph are however, not so marked, as may be seen from Table II, in which details of some typical results are given.

Samples *A* and *B* are soft wheat blends, while *C* and *D* are hard wheat blends. In each case the production of carbon dioxide from the commercially milled flour follows fairly closely that produced

TABLE II

DIASTATIC ACTIVITY AND GASSING POWER OF CORRESPONDING EXPERIMENTALLY AND COMMERCIALY MILLED FLOURS

Fermentograph Gassing Power—C.Cs. CO₂ Produced per Hour

Sample	A		B		C		D	
Flour	Experi- mental	Com- mercial	Experi- mental	Com- mercial	Experi- mental	Com- mercial	Experi- mental	Com- mercial
1st hour	330	340	410	400	270	290	330	320
2nd hour	460	410	470	460	420	370	460	420
3rd hour	540	490	605	600	410	420	540	470
4th hour	570	490	620	580	470	450	530	460
5th hour	490	420	520	505	400	380	470	400
6th hour	380	345	310	315	350	340	420	360
7th hour	230	245	190	190	320	270	360	265
5-hour total	2390	2150	2625	2545	1970	1910	2330	2070
7-hour total	3000	2740	3125	2750	2640	2520	3110	2695
Diastatic activity—mg. maltose per 10 g. flour (Kent-Jones method)	237	209	248	218	384	248	426	287

from the experimentally milled flour, but is nearly always just a little below in value. As the samples are blends they can not be strictly compared with the previous results which are from definite varietal samples, but serve to indicate the characteristics of the commercial flour corresponding to these samples.

From a limited number of analyses it would seem that the original sucrose value of commercially milled flours from soft wheats is also below that of corresponding flours milled on the Brabender mill by approximately 30 mg. per 10 g. of flour. This figure is only tentative and may be altered after more analyses have been carried out. No determinations have yet been made on commercial flours from hard wheats.

Results of Diastatic Activity Measurements

Two or more determinations of diastatic activity were made on each sample both with the Kent-Jones and Blish-Sandstedt methods. The average results are shown in Table III together with the total

TABLE III
DIASTATIC ACTIVITY AND GASSING POWER

Sample number	Mg. maltose per 10 g. flour		C.c. of CO ₂ (Fermentograph method)	
	Kent-Jones method	Blish-Sandstedt method	After 5 hours	After 7 hours
1	170	148	1970	
2	449	383	2040	2770
3	227	208	1810	
4	366	300	2590	3140
5	351	293	2590	3460
6	197	220	1990	2680
7	112	107	1490	
8	154	157	1910	
9	167	198	1970	2590
10	111	115	1670	
11	185	220	2250	2805
12	348	458	1890	2460
13	188	198	2320	2860

amount of carbon dioxide produced in five and in seven hours in the Fermentograph test. The results are based on flour at 15.0% moisture content since it was approximately at this moisture content that the tests were carried out.

In Table IV the samples have been listed in the order of their diastatic activity, while in Table V are given the results of analysing standard sugar solutions by the Kent-Jones and Blish-Sandstedt methods.

TABLE IV
ORDER OF DIASTATIC ACTIVITY AND GASSING POWER OF SAMPLES

Order	Diastatic activity ¹		Gassing power	
	By Kent-Jones method	By Blish-Sandstedt method	By Fermentograph C.c. of CO ₂	
			5-hour total	7-hour total
1st	No. 2. (449)	No. 12. (458)	No. 4. } (2590)	No. 5. (3460)
2nd	4. (366)	2. (383)	5. }	4. (3140)
3rd	5. (351)	4. (300)	13. (2320)	13. (2860)
4th	12. (348)	5. (293)	11. (2250)	11. (2805)
5th	3. (227)	6. }	2. (2040)	2. (2770)
6th	6. (197)	11. } (220)	6. (1990)	6. (2680)
7th	13. (188)	3. (208)	1. }	9. (2590)
8th	11. (185)	13. }	9. } (1970)	12. (2460)
9th	1. (170)	9. } (198)	8. (1910)	
10th	9. (167)	8. (157)	12. (1890)	
11th	8. (154)	1. (148)	3. (1810)	
12th	7. (112)	10. (115)	10. (1670)	
13th	10. (111)	7. (107)	7. (1490)	

¹ Milligrams of maltose per 10 g. of flour.

TABLE V
COMPARATIVE TESTS OF STRENGTH OF MALTOSE SOLUTIONS

(Maltose values expressed as milligrams maltose per 10 g. flour. All results are the mean of two or more determinations.)

Theoretical maltose value	Kent-Jones method		Blish-Sandstedt method	
	Mg. of maltose	% of theoretical	Mg. of maltose	% of theoretical
150 (Buffered solution)	197.5	98.8	141	94.1
200 (Buffered solution)			182	91.0
350 (Buffered solution)			318	90.8
150 (Unbuffered solution)	199.7	99.9	126	84.0
200 (Unbuffered solution)			168	84.0
296 (Unbuffered solution)			256	86.5
400 (Unbuffered solution)			340	85.0

The buffered solutions used in the above analyses were made as follows: 2.5 g. of B.D.H. maltose were dissolved in water to give a 1.0% solution. To 50 ml. of this solution there were added 180 ml. of buffer solution, 10 ml. of 10% H₂SO₄ and 10 ml. of 12% tungstate solution.

The other solutions were made up in a similar manner, proportional amounts of buffer and acid solutions, *etc.*, being used. They were then tested as for flour extracts. (See Davis, 1937.)

The flours were also tested for original sucrose content by inverting an aliquot of the water extract with hydrochloric acid, diluting to half the original concentration and then titrating against Fehling's solution (Kent-Jones, 1927).

The results of the sucrose estimations are given in Table VI.

TABLE VI
SUCROSE IN FLOURS (MILLIGRAMS PER 10 G. FLOUR)

Sample	Sucrose (mean of determinations)
1. Ranee, Vic.	269
2. Dundee, Vic.	218
3. Nabawa, N.S.W.	229
4. Baringa, N.S.W.	269
5. Pusa, N.S.W.	222
6. Ranee, S.A.	317
7. Ford, S.A.	198
8. Nabawa, S.A.	249
9. "Selection 28," S.A.	268
10. Ford, S.A.	170
11. Ranee, S.A.	272
12. Nabawa, W.A.	283
13. Bencubbin, W.A.	317

In order more easily to relate the gassing power with maltose and sucrose contents, the hourly gas production for each sample has been given in Table VII together with these figures.

TABLE VII
DIASTATIC ACTIVITY, SUCROSE CONTENT AND CO₂ PRODUCTION PER HOUR

Sample	Dia- stastic activity Kent- Jones method	Orig- inal su- crose	CO ₂ produced—c.c. per hour							Total CO ₂ (c.c.) produced	
			(Milligrams per 10 g. flour)	1.	2.	3.	4.	5.	6.	7.	After 5 hours
1. Ranee	170	269	370	400	460	470	270	150	—	1970	
2. Dundee	449	218	260	340	460	520	460	390	340	2040	2770
3. Nabawa	227	229	260	400	470	450	230	—	—	1810	
4. Baringa	366	269	240	440	580	650	680	370	180	2590	3140
5. Pusa	351	222	230	400	600	680	680	560	310	2590	3460
6. Ranee	197	317	300	420	440	440	390	380	300	1990	2680
7. Ford	112	198	300	350	460	260	120	—	—	1490	
8. Nabawa	154	249	350	370	480	440	310	190	—	1910	
9. "Selection 28"	167	268	350	430	460	380	350	340	280	1970	2590
10. Ford	111	170	320	380	480	270	120	—	—	1670	
11. Ranee	185	272	320	405	520	550	460	350	205	2250	2805
12. Nabawa	348	283	370	410	410	340	340	290	280	1890	2460
13. Bencubbin	188	317	390	560	500	470	400	330	210	2320	2860

Discussion

From Table III it will be seen that there is not a constant relationship between the two methods used for the determination of the reducing sugar after diastasis, for the Blish-Sandstedt figures range from 66 mg. (Nos. 2 and 4) below, to 110 mg. (No. 12) above those obtained by the Kent-Jones method. Neither method shows a good correlation with the Fermentograph results (see Table IV) which, from both practical and theoretical considerations, are the more likely results to be obtained in baking practice.

Assuming therefore that the order based on Fermentograph results in which the samples are placed is correct, it will be seen from Table IV, or perhaps more easily from Table VIII, that both methods used

TABLE VIII
ORDER OF DIASTATIC ACTIVITY AND GASSING POWER OF SAMPLES
(Table IV Showing Actual Varieties)

Order based on diastatic activity		Order based on gassing power
Kent-Jones method	Blish-Sandstedt method	Fermentograph (5-hour total)
No. 2. Dundee, Vic. 4. Baringa, N.S.W. 5. Pusa, N.S.W. 12. Nabawa, W.A. 3. Nabawa, N.S.W. 6. Rane, S.A. (T) 13. Bencubbin, W.A. 11. Rane, S.A. (R) 1. Rane, Vic. 9. Selection 28, S.A. 8. Nabawa, S.A. (T) 7. Ford, S.A. (T) 10. Ford, S.A. (R)	No. 12. Nabawa, W.A. 2. Dundee, Vic. 4. Baringa, N.S.W. 5. Pusa, N.S.W. { 11. Rane, S.A. (R) 6. Rane, S.A. (T) 3. Nabawa, N.S.W. { 9. Selection 28, S.A. 13. Bencubbin, W.A. 8. Nabawa, S.A. (T) 1. Rane, Vic. 10. Ford, S.A. (R) 7. Ford, S.A. (T)	No. 4. Baringa, N.S.W. } 5. Pusa, N.S.W. } 13. Bencubbin, W.A. 11. Rane, S.A. (R) 2. Dundee, Vic. 6. Rane, S.A. (T) { 1. Rane, Vic. 9. Selection 28, S.A. 8. Nabawa, S.A. (T) 12. Nabawa, W.A. 3. Nabawa, N.S.W. 10. Ford, S.A. (R) 7. Ford, S.A. (T)

for the determination of reducing sugars after diastasis show about the same degree of misplacements as compared with the Fermentograph results (5-hour totals). The discrepancies between actual gassing power and expected gassing power as judged from the diastatic activity measurements are not uniform, the actual gassing power being sometimes above (e.g., Bencubbin W.A. sample) and sometimes below (e.g., Nabawa W.A. and Dundee Vic. samples) the values expected. This variation cannot be attributed to grinding effects on wheats of different physical characteristics. Rather does it illustrate the conclusions reached by Sandstedt, Blish, Mecham, and Bode (1937)

that some flours probably contain a small amount of "available" starch together with an enzyme or "activator" that is responsible for rendering the raw starch available to the sugar-producing enzyme, beta-amylase. The content of this particular enzyme or "activator" varies from flour to flour, apparently being greater in the hard than in the soft wheat flours. In the diastatic activity test the length of time involved is not sufficient for this enzyme to become significant, *i.e.*, sugar is still being produced from the available starch, consequently there is no indication of its activity or concentration as there is in the gassing power test.

From the results given in Table V, obtained by analysing standard sugar solutions by the two different methods under consideration, it will readily be seen that the Kent-Jones method has a lower experimental error. Its error only varies 1.0% (from 99.9 to 100.9%) over a range of maltose values from 150 to 400 mg. per 10 g. of flour while the Blish-Sandstedt method shows a variation in the experimental error of 3.3% (90.8 to 94.1%) over a range of 150 to 350 mg. maltose per 10 g. flour, the error increasing with increasing concentration of maltose. The results also demonstrate the need for correct buffering of the sugar solutions when the latter method is employed.

When it was seen that the correlation between diastatic activity and gassing power was rather irregular, determinations of original sucrose were carried out on the test samples.

The results (see Table VII) varied rather widely and were mainly remarkable for their high value. They show how it is possible for a flour with a low diastatic activity figure, such as sample 13, to produce when fermented in a dough more carbon dioxide than would be expected from a consideration of its diastatic activity alone. On the other hand, sample 12 has both a high diastatic activity and initial sucrose content and yet only ranks 10th on its gassing power (5-hour total) thus indicating that a third factor is involved in gas production. This factor we presume to be the enzyme or "activator" discussed above.

The grouping of the samples according to the Fermentograph test (gas produced per 5 hours) is noteworthy because although only a limited number of samples have been considered, they have, apart from one exception (No. 2 Dundee), fallen into varietal groups (Table VIII).

The amount of gas produced by the samples in 5 hours by the Fermentograph method has been used for comparative purposes because it is practically more significant than the 7-hour gas-production figure since the yeast-salt ratio was chosen for a 5-hour dough.

Summary and Conclusions

A comparison has been made, from the point of view of routine mill control, of the relationship between diastatic activity, as measured by the Kent-Jones modification of Rumsey's method and by the Blish-Sandstedt method, and gassing power, using the Brabender Fermentograph, of flours from different Australian varieties of wheats which have been milled on a Brabender experimental mill. Although the number of wheat samples considered here is limited the analytical results, in the author's opinion, are fairly indicative of the particular varieties examined unless otherwise stated. Sucrose estimations have been made and a comparison has been drawn between flours from wheats milled on the Brabender experimental mill and their corresponding commercial flours.

The conclusions are as follows:

Measurement of diastatic activity. A poor correlation was obtained between the Blish-Sandstedt and Kent-Jones methods for measuring reducing sugars after diastasis, the difference between them varying from positive to negative in an inexplicable manner.

The Kent-Jones method has a lower experimental error and is more suited to routine work than the Blish-Sandstedt method since it is faster, more economical in chemicals, and there is less possibility for mistakes in technique.

Correlation between diastatic activity and gassing power. The correlation between diastatic activity and gassing power is on the whole rather poor though it is slightly better with the Blish-Sandstedt than with the Kent-Jones method, but not sufficiently so to outweigh the advantages outlined above of the Kent-Jones method.

Since diastatic activity measurements do not account for all the factors which influence gas production, the results so obtained must necessarily be less informative than methods which actually measure gas production.

The results obtained in this experiment show that whereas a diastatic activity measurement may give some indication of the probable gassing power of a flour, it may frequently be misleading and should always be supplemented by a gassing power test in the case of flours from "unknown" wheat samples.

The main use of diastatic activity measurements is in mill-control work when the type of flour under consideration varies only within narrow limits.

The Brabender Fermentograph affords a logical, easy and accurate method for the measurement of gassing power.

Varieties considered. The number of samples examined is too small to make definite statements regarding the gassing power of different varieties but the indications are definitely that the gassing power is a varietal characteristic which, unlike "strength," tends to be of the same order irrespective of the district in which the sample is grown, *e.g.* Ranee, Nabawa and Ford samples. The hard wheats, Pusa and Baringa, are most vigorous in gassing power while the Ford variety is undoubtedly the poorest. The Ford samples had the lowest diastatic activity as determined by both the Blish-Sandstedt and the Kent-Jones methods, the lowest sucrose content and the lowest gassing power.

Experimental and corresponding commercial flours. Flours from wheats milled on the Brabender experimental mill show higher diastatic activity figures and higher gassing power than corresponding commercial flours though the Fermentographs show less difference than do the diastatic activity figures. Typical relationships have been set out in Table II.

Acknowledgment

The author wishes to express his appreciation to the donors of the samples used in this experiment, and to W. S. Kimpton & Sons for permission to publish this report.

Literature Cited

- American Association of Cereal Chemists
1935 Cereal Laboratory Methods, Third Edition, pp. 56-58. Published by the A. A. C. C.
- Davis, C. F.
1937 A collaborative study of the Blish-Sandstedt, Schoorl, and Bertrand methods for determining reducing sugars in flour diastatic activity measurements. Cereal Chem. **14**: 74-85.
- and Worley, D. F.
1934 The correlation between diastatic activity and gassing power in commercial flours. Cereal Chem. **11**: 536-545.
- Kent-Jones, D. W.
1927 Modern Cereal Chemistry, pp. 347-357.
- Sandstedt, R. M., Blish, M. J., Mecham, D. K., and Bode, C. E.
1937 Identification and measurement of factors governing diastasis in wheat flour. Cereal Chem. **14**: 17-34.

STUDIES ON THE STORAGE OF WHEATEN FLOUR:
III. CHANGES IN THE FLORA AND THE
FATS AND THE INFLUENCE OF
THESE CHANGES ON
GLUTEN CHARACTER

E. C. BARTON-WRIGHT ¹

The Research Association of British Flour-Millers, St. Albans, England

(Received for publication December 22, 1937)

A continuation of the previous investigations on the changes that take place in flours during storage carried out in these laboratories (Fisher, Halton, and Carter, 1937) has fully confirmed earlier results. It was shown in the earlier investigations that marked chemical changes occur in flour kept under storage conditions over a prolonged period of time. It was suggested that perhaps biological agencies such as bacteria and fungi, and possibly mites, may have been responsible for them. The earlier investigations were entirely chemical in nature and the determinations made then have been repeated, together with certain additional analyses. At the same time a biological examination has been carried out to ascertain whether the bacterial and fungal flora of flour show any marked changes in nature and number during storage, which could be correlated with the chemical changes that have been observed.

The only systematic bacteriological investigation of flour of which we are aware is that carried out by Kent-Jones and Amos (1930). It was shown by these investigators that the bacterial numbers of different flours vary enormously. For example, it was found by them that in a patent flour the bacterial numbers may be as high as 70,000 per gram, while in low-grade flours still higher figures were recorded, up to 600,000 per gram, whereas straight runs were found to occupy a somewhat intermediate position, up to 120,000 per gram. It was further discovered by Kent-Jones and Amos that on storage the bacterial numbers decreased. Flours stored for as short a period as 26 days showed a fall of over 50% over the initial values, while after storage for 70 days, the value per gram of flour fell even further.

In the present investigation, the preliminary storage experiments were carried out with two different low-grade flours, an English (bot-

¹ The author wishes to express thanks to E. A. Fisher (Director of Research) for suggesting this investigation and for his interest and valuable suggestions while the work was in progress.

tom 40%) and Manitoba (bottom 50%). These were stored in tins at two different moisture contents. The English flour had an initial moisture content of 16.48% and the Manitoba 14.56%. A portion of each of these flours was moistened to approximately 18%. Samples of the flours were removed at fortnightly intervals for the various routine analyses and bacterial and fungal counts.

For the bacterial counts, and also later, for the fungal counts, the technique originally devised by Kent-Jones and Amos proved to be very suitable. In this method 100 c.c. of a 0.5% sterile saline solution are placed in a wide-necked sterile glass bottle and 10 g. of freshly ignited sand added. Ten grams of the sample of flour to be investigated are accurately weighed out in a sterile dish and added to the saline-sand mixture and the whole shaken for 2 minutes. The mixture is allowed to settle and 5 c.c. are pipetted into 45 c.c. of sterile saline solution which is also contained in a wide-necked sterile glass bottle. This is now shaken for 1 minute, and after the mixture has settled a 5 c.c. aliquot is pipetted into a further 45 c.c. of sterile saline solution. Finally, 1-c.c. lots are pipetted out of this last dilution into sterile petri dishes for the plating out. This degree of dilution was found sufficient in the great majority of cases for the bacterial and fungal numbers encountered in the different flours used in this work.

In the preliminary stages of the investigation, nutrient beef or Lemco agar and gelatin were used for the plating out. Later, however, both beef and Lemco agar and gelatin were abandoned and the synthetic medium devised by Thornton (1922) was employed instead. The Thornton medium has the following composition:

K_2HPO_4	1.0	g.
$MgSO_4 \cdot 7H_2O$	0.2	g.
$CaCl_2$	0.1	g.
$NaCl$	0.1	g.
$FeCl_3$	0.002	g.
KNO_3	0.5	g.
Asparagine	0.5	g.
Mannitol	1.0	g.
Agar	15.0	g.
Water	1000	c.c.

To make up the medium, phosphate, nitrate and asparagine are dissolved in the same sample of water in the order named, and the $MgSO_4$, $CaCl_2$, $NaCl$ and $FeCl_3$ in separate quantities of water which are then added to the first solution in the order given above. Agar is next added and dissolved at 100° C. The temperature is allowed to fall to 60° C. the mannitol is added, and the pH of the medium adjusted to 7.4 with N/10 NaOH. It is now "tubed" and sterilised at 15 pounds pressure for 15 minutes.

For the fungal counts a synthetic medium was also employed made up as follows:

K ₂ HPO ₄	0.5 g.
Asparagine	1.0 g.
Glycerol	10.0 c.c.
Agar	15.0 g.
Water	1000 c.c.

The agar is dissolved at 100° C. and the pH adjusted to 4.8 with N/10 HCl. The medium is "tubed" and sterilised at 15 pounds pressure for 15 minutes.

With either Lemco or beef extract media it is impossible, owing to variations in the material, to obtain absolutely standard batches of cultures. On this account variations in bacterial numbers obtained on such media may well be due to variations in the composition of the medium. With a synthetic medium, such as that described above, it is possible to prepare each fresh batch of medium under completely standard conditions, and variations in bacterial or fungal numbers due to variations in the composition of the medium are eliminated. There is also the further fact that the presence of "spreaders," such as *Bacillus dendroides*, prevents accurate counts being made when a Lemco or beef extract nutrient medium is used. Their presence is very largely obviated when a synthetic mineral medium is employed.

For the fortnightly counts of both bacterial and fungal numbers, 10 plates of each medium (bacterial and fungal) were plated out and incubated at 25° C. for 7 days. In all cases χ^2 was calculated for each set of samples from the equation:

$$\chi^2 = \frac{S(x - \bar{x})^2}{\bar{x}},$$

where S stands for the summation over the whole sample, $\bar{x} = \frac{1}{n}S(x)$, n is the number of degrees of freedom and $\bar{x}$ is the mean of a number of observations. The conception of χ^2 is extremely important in connection with bacterial and fungal counts in estimating the numbers of these organisms by the dilution method, since it is possible by the use of this value to ascertain whether the distribution of the organisms follows the Poisson Series. An example of such a distribution as the Poisson Series is given by counts of yeast and bacterial cells in a culture where the distribution is necessarily discontinuous. It is possible by using the above equation and the tables given by Fisher (1934) to calculate significant deviations from expectation.

In neither of the two samples of flour (English and Manitoba) nor in any of the subsequent flours that were examined for bacterial

numbers, were the high figures recorded by Kent-Jones and Amos obtained. The highest figure recorded by us was 57,000 per gram.

The most important changes, biological and chemical, were found to occur, as would be expected, in the moistened samples. Here there was a rapid increase in soluble nitrogen (*i.e.*, soluble protein) and decrease in hydrogen-ion concentration, a fall in bacterial and an increase in fungal numbers. For example, the initial counts of bacteria at the commencement of storage (7.4.35) gave for the English low-grade sample at normal moisture content 40,750 per gram, and the corresponding value for the Manitoba sample was 37,800 per gram. Fungal counts were not taken at this time as a suitable technique had not been developed. Ten weeks later the bacterial numbers for the English normal sample were 14,400 per gram and the English moist sample 4,200 per gram, whereas the normal Manitoba sample gave 14,150 and the Manitoba moist sample 3,450 per gram. The fall in the bacterial numbers recorded for these samples corresponds with a decrease in the hydrogen-ion concentration over the same period of time, as may be seen from the figures given in Table I.

TABLE I
CHANGES IN pH AND BACTERIAL CONTENT OF FLOUR DURING STORAGE

English normal			English moist		
Period of storage in weeks	pH	Bacterial numbers per gram	Period of storage in weeks	pH	Bacterial numbers per gram
0	6.18	40,750	0	6.18	40,750
4	6.18	40,342	4	6.15	26,400
6	6.18	35,000	6	6.13	15,000
8	6.16	14,800	8	5.66	6,321
10	5.66	14,400	10	4.93	4,200
Manitoba normal			Manitoba moist		
0	6.24	37,800	0	6.24	37,800
4	6.27	37,600	4	5.93	14,800
6	6.21	25,500	6	5.17	4,500
8	6.19	14,856	8	5.14	3,900
10	6.14	14,150	10	5.33	3,450

It is a well known fact that bacteria do not multiply rapidly in a medium of less than pH = 6.0 and it is interesting to compare the rapid increase in hydrogen-ion concentration in the moist samples during storage with the especially great fall in bacterial numbers in the same samples.

From a large number of fungal counts on different flours, it has been found that the average initial values in freshly milled normal flours,

i.e., excluding very low-grade flours, lie between 1,000 and 2,000 per gram. In the moist sample of Manitoba flour, the fungal content rose from an initial value of 1,200 per gram to 8,280 per gram in 10 weeks, and the odour of mould was noticed after 8 weeks of storage. After 30 weeks of storage the fungal numbers had increased to 24,400 per gram and the bacterial numbers had fallen to 1,000 per gram. A marked increase in the fungal numbers was also observed in the moist English sample.

Over 90% of the fungi isolated from flours belong to the genus *Penicillium*, but other genera are also present, *e.g.*, *Botrytis*, *Aspergillus* and *Cladosporium*, as well as certain unidentified forms. The unidentified genera were examined at our request by S. F. Ashby, Director of the Imperial Mycological Institute, Kew, who suggested that they were very similar to certain forms that had been isolated from frozen meat by Brooks and Hansford (1922). Of the *Penicillia* present, *P. brevicompactum*, which forms mainly mycophenolic acid ($C_{17}H_{20}O_6$) from glucose in Roulin-Thom's medium, was fairly abundant, as well as a number of other species which also gave rise to mycophenolic acid, *e.g.*, *P. patris-mei* Zaleski. By far the greater number of *Penicillia* isolated from flour gave rise, however, on Roulin-Thom's medium to polymannose and polygalactose. A description of these products of fungal metabolism was first given by Clutterbuck *et al.* (1934) for a *Penicillium* isolated by them from mouldy maize, and to which they gave the specific name of *P. Charlesii*. This form is said by them to belong to the Monoverticillata-Ramigena group of Thom (1930). A number of *Penicillia* have been isolated by us from flour which give rise to these two polysaccharides, but which do not belong to this group. Some of these have been named for us. For example, one *Penicillium* (our laboratory number N.U. 17) was found to belong to Thom's Fasciculata-Viridicata group, while another (N.U. 18) was found to be identical with *Penicillium Expansum* (Thom's series) and yet another (N.U. 32) to Thom's *Aeruginosa* group and the nearest species with which it could be identified was *P. cyclopium* Westling. All these forms gave rise to polymannose and polyglucose when grown on Roulin-Thom's medium.

The fact that bacteria tend to disappear, and the numbers always fall to a very low value during prolonged storage of flour, whereas fungi increase rapidly in numbers if the moisture content of the flour be 16.0% or higher, explains why bacterial troubles are rarely encountered, if ever, during prolonged storage of flour. On the other hand, fustiness and mustiness due to mould growth are well known.

In the moistened samples of flour, the oil content (fraction soluble in petrol ether) was found to decrease rapidly during storage. The